Virchows Arch [Pathol Anat] (1981) 393:205–214

Virchows Archiv A

Cell Kinetic and Cytological Grading of Prostatic Carcinoma

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Summary. In 69 cases of prostatic carcinomas the ^{3}H-thymidine labelling index was studied, and a cytological analysis was performed in addition to the histological classification. The following cytological variables were measured: nuclear size and shape, nuclear-cytoplasmic-ratio, nuclear chromasia, size and form of nucleoli.

Only prostatic carcinomas with a labeling index of less than 0.4% showed mild nuclear anaplasia. These carcinomas were histologically classified as well differentiated adenocarcinomas. Prostatic carcinomas with a labelling index from 0.4% to 1.0% showed moderate nuclear anaplasia. These carcinomas were poorly differentiated adenocarcinomas. Carcinomas with labeling indices between 1.4 and 2.3% had moderate – marked nuclear anaplasia and exhibited a transition to undifferentiated carcinomas. Carcinomas with a labelling index higher than 2.3% were cribriform and undifferentiated carcinomas with marked nuclear anaplasia.

The present studies have shown a correlation between both histological classification and cytological appearances with indicators of cell kinetics.

Key words: Prostatic carcinoma – Histology – Cytology – Autoradiography – Grading

Introduction

The histological grading of prostatic carcinoma is important in evaluation of prognosis and choice of therapy. The grading depends on the histological and cytological pattern. The most common prostatic carcinomas are classified histologically into well and poorly differentiated adenocarcinomas, cribriform and undifferentiated solid anaplastic carcinomas. The cytological grading into the grades I, II, III, is dependent on the degree of nuclear anaplasia (Gleason

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0340-1227/81/0393/0205/$02.00

1966; Mostofi 1975, 1980; Dhom 1977, 1978; Murphy and Whitmore 1979; Böcking and Sinagowitz 1980; Müller et al. 1980).

It is most difficult to classify borderline cases between well and poorly differentiated adenocarcinomas. The exact histological and cytological diagnosis of well differentiated adenocarcinomas, especially of incidental carcinomas, is important for prognosis (Mellinger et al. 1967; Esposti 1971; Gleason et al. 1974; Dhom and Hautumm 1975; Epstein 1976; Schröder et al. 1978). Patients with well differentiated adenocarcinomas show a significantly higher probability of surviving (10–15 years) than patients with cribriform and undifferentiated carcinomas. Nuclear anaplasia also correlates well with mortality in prostatic carcinomas (Faul et al. 1978). The probability of survival in patients with prostatic adenocarcinomas showing mild anaplasia hardly differs from age-matched healthy males (Mostofi 1975; Dhom 1977, 1978; Böcking et al. 1979).

Furthermore, therapeutical measures may be chosen depending on the histological and cytological grading. The effect of therapy is often evaluated by cytological grading (Schubert et al. 1973; Alken et al. 1975, 1977; Faul et al. 1978; Leistenschneider and Nagel 1980).

In recent studies the grade of nuclear anaplasia was studied in each type of prostatic carcinomas. The grade of anaplasia was divided into three types mild, moderate and marked (Böcking and Sinagowitz 1980). In well differentiated adenocarcinomas, tumors with mild nuclear anaplasia occur as frequently as those with moderate anaplasia, indicating that further cytological differentiation might be possible.

In previous autoradiographic studies we established a correlation between the histological type of carcinoma before and during hormonal treatment and the proliferative activity, finding that less differentiated carcinomas showed higher labelling and mitotic indices (Helpap et al. 1974, 1976). The present study deals with the relationship between cytological characteristics and proliferative activity.

Methods

69 tissue biopsies of prostate carcinomas, obtained with a TRU cut biopsy needle (Travenol), were examined. The biopsies were incubated in autologous plasma or synthetic medium (TCM) with tritiated thymidine (^{3}H-TdR 5.0 µCi/ml. specific activity 20.0 Ci/m mol NEN Chemicals, Boston Mass. USA). During the incubation the carbogen pressure was 2.2 atm and the temperature 37° C (Helpap et al. 1974).

After embedding in paraplast, stripping film autoradiographs (AR 10 Kodak) were made in the usual manner. The exposure time was 30 days. In the autoradiographs stained with haematoxylin the percentage of radioactively labelled nuclei of the tumor cells (labelling index) was determined. On the average 1,000 cells per slide were counted (Helpap et al. 1976).

The carcinomas were classified histologically into well and poorly differentiated adenocarcinomas, cribriform and undifferentiated solid anaplastic carcinomas according to the German Prostate Cancer Registry, Homburg/Saar (Dhom 1977).

Using a counting eyepiece graticule (Leitz) the cytological evaluation was performed under a high magnification (1,000 ×). In five different tissue area units of 0.0064 mm^2 we scored the following cytological variables (Fig. 1):

1. Nuclear size: For each specimen the relative number of cells with equally sized nuclei was calculated allowing a distinction between one to four classes (a, b, c, d). These classes were

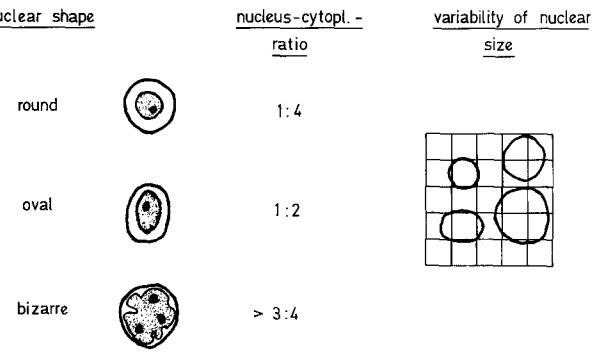

Fig. 1. Counting of nuclear size and shape, nuclear-cytoplasmic-ratio and number of nucleoli in prostatic carcinomas with an eyepiece graticule

distributed by their frequency. In Fig. 3 "a" indicates the predominant cell group, "d" the one which occurs least frequently.

2. Nuclear shape (round, oval, bizarre)
3. Nuclear chromasia (mild, moderate, marked)
4. Nuclear-cyto-plasmic-ratio (0.25, 0.5, 0.75, more than 0.75)
5. Number of nucleoli
6. Nucleolus-nucleus-ratio (less or more than 0.125)

We plotted the percentage distribution of the different cytological variables against the labeling indices of the prostatic carcinomas. The standard deviation was given as $2\,s = 95\%$ probability.

Results

Combined Histological and Cytological Analysis of Prostatic Carcinomas

Well differentiated adenocarcinomas showed predominantly round nuclei with mild nuclear chromasia (Fig. 2a). A nuclear-cytoplasmic-ratio of more than 0.25 and the presence of several nucleoli were characteristic of poorly differentiated adenocarcinomas (Fig. 2b). In cribriform carcinomas there was significant nuclear polymorphism with numerous prominent nucleoli (Fig. 2c). Undifferentiated carcinomas had the highest grade of nuclear anaplasia (Fig. 2d).

Combined Cell Kinetic and Cytological Analysis of Prostatic Carcinomas

69 carcinomas were analysed autoradiographically. According to the labelling indices which ranged between 0.1 and 5.6%, the cases of prostatic carcinomas were divided into 13 groups as shown in Table 1.

Nuclear Size. Prostatic carcinomas with a labelling index less than 0.4% showed predominance of a group of cells with similar nuclear size (group "a" in Fig. 3). In carcinomas with a labelling index exceeding 0.5% cell groups with different nuclear size were found, i.e. the degree of polymorphism had increased (Fig. 3).

Nuclear Shape. The nuclear shape also correlated with the proliferative activity. Round nuclei predominated in the carcinomas, with a labelling index less than

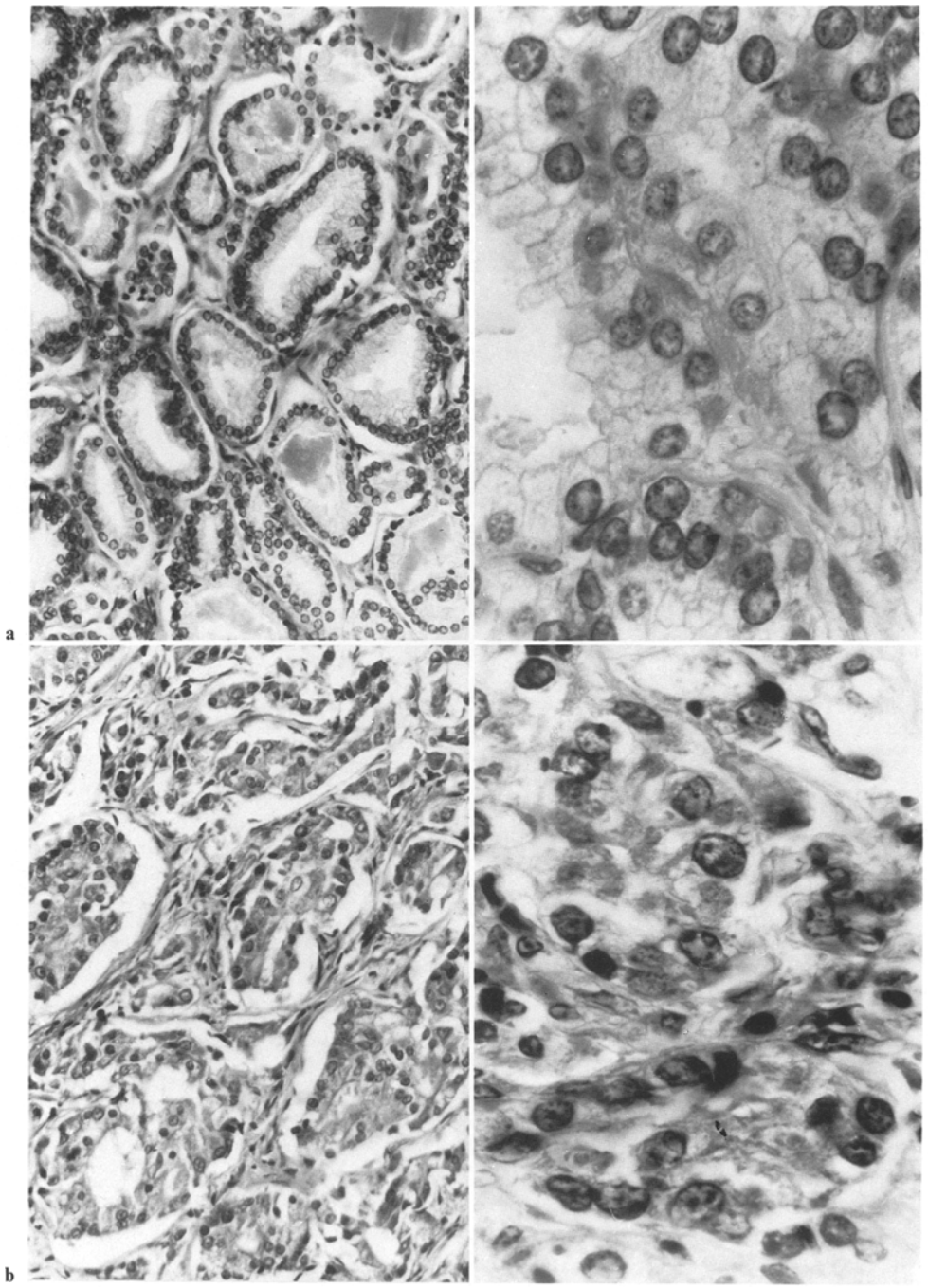

Fig. 2a–dd. Histological pattern and cytological details of prostatic carcinomas. **a** Well differentiated adenocarcinoma. HE ×310. **aa** Cytologically round nuclei of similar size and mild chromasia. HE ×1,024. **b** Poorly differentiated adenocarcinoma. HE ×310. **bb** Increased nuclear polymorphism and chromasia. Several nucleoli (moderate to marked anaplasia). HE ×1,024. **c** Cribriform carcinoma. HE ×310. **cc** Numerous prominant nucleoli. HE ×1,024. **d** Undifferentiated carcinoma. HE ×310. **dd** Marked nuclear anaplasia. HE ×1,024

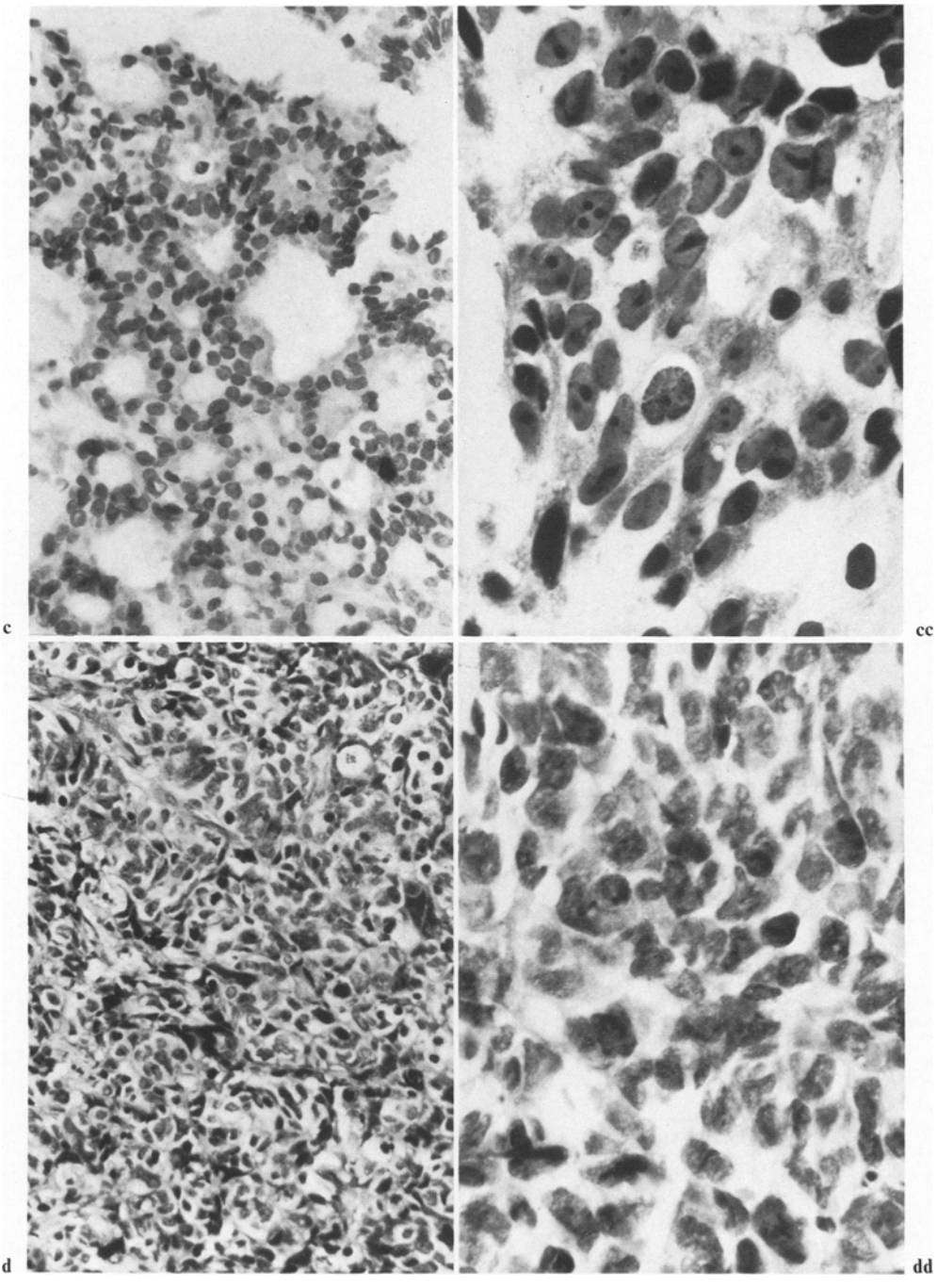
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Table 1. Autoradiographic findings (labelling index) in prostatic carcinomas

Histological classification	*n*	Labelling index (%)
Well differentiated adenocarcinoma	7	0.1
	10	0.3
	9	0.4
Well and poorly differentiated adenocarcinoma	8	0.5
Poorly differentiated adenocarcinomas	6	0.6
	4	0.7
	3	0.8
	4	1.0
	4	1.4
Cribriform and undifferentiated carcinomas	5	2.3
	3	3.5
	4	4.4
	2	5.6

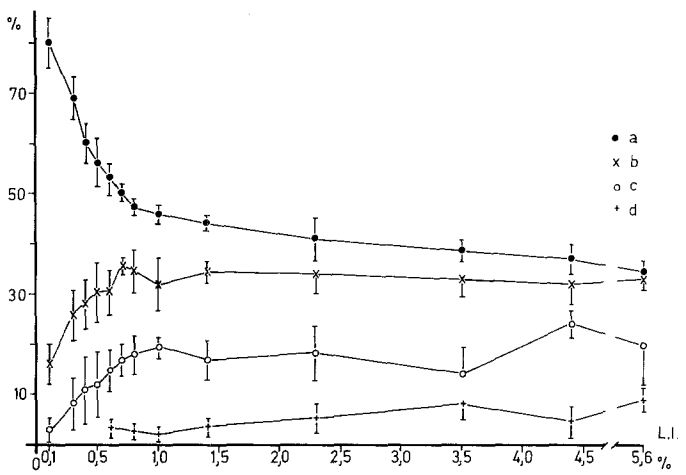

Fig. 3. Percentage distribution of different classes of nuclear size (*a, b, c, d*) to the labelling index of prostatic carcinoma. The relative number of cells with equally sized nuclei was calculated allowing the distinction between one to four classes. "a" indicates the predominant cell group, "d" the one which occurs least

0.4%. Carcinomas with an average labelling index of 0.88% had equal amounts of round, oval and bizarre nuclei. Carcinomas with a labelling index exceeding 1.0% the nuclear shape was oval, later on bizarre (Fig. 4).

Nuclear Chromasia. A mild nuclear stainability was observed in carcinomas with a labelling index less than 0.5%. Carcinomas with a labelling index of 0.6–0.7% showed similar frequencies of nuclei with mild, moderate and marked chromasia. A strong hyperchromasia predominated in carcinomas with a labelling index above 0.8% (Fig. 5).

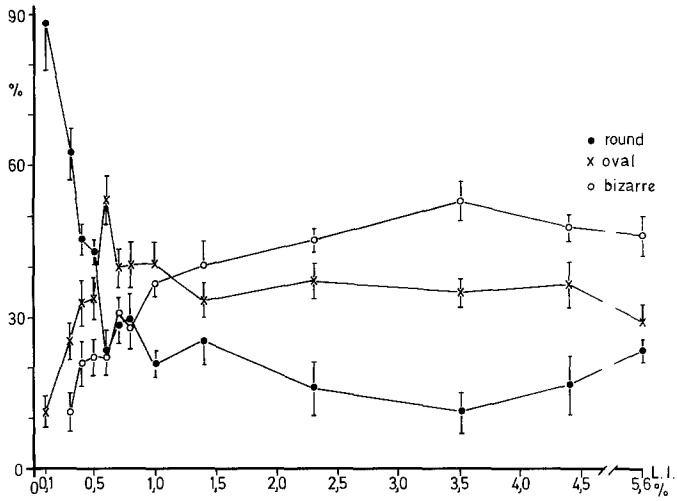

Fig. 4. Relation of nuclear shape and labelling index of prostatic carcinomas

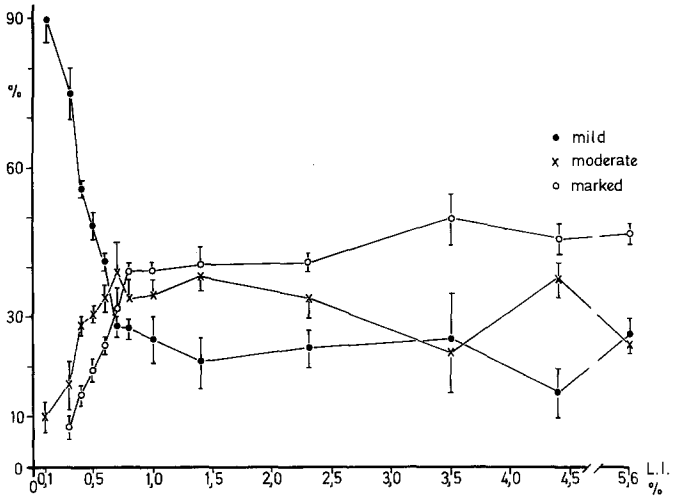

Fig. 5. Correlation of heterochromasia and labelling index of prostatic carcinomas

Nuclear-Cytoplasmic-Ratio. A ratio of 0.25 occurred only in carcinomas with a labelling index less than 0.3%. The nuclear-cytoplasmic ratio increased up to 0.75 when the labelling index rose to 3.5%. The ratio became higher than 0.75 in prostatic carcinomas with a labelling index more than 3.5% (Fig. 6).

Number of Nucleoli and Nucleolus-Nucleus-Ratio. Prostatic carcinoma cells with one nucleolus and with a nucleolus-nucleus-ratio of 0.125 were predominant in carcinomas with a labelling index less than 0.3%. With an increase of labelling indices the tumor cells showed a tendency to have several nucleoli and the nucleolus-nucleus-ratio rose to more than 0.125.

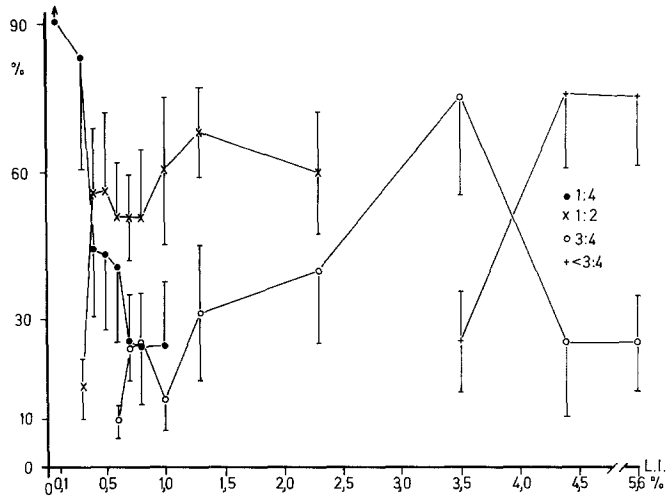

Fig. 6. Relation of nuclear-cytoplasmic-ratio and labelling index of prostatic carcinomas

Discussion

Cell kinetic analysis has shown that the labelling index of adenocarcinomas ranged between 0.1 and 1.4%. Only adenocarcinomas with a labelling index less than 0.4% showed mild nuclear anaplasia. In these instances it may be assumed that the carcinomas have decreased proliferative activity and in this sense a diagnosis of "well differentiated adenocarcinomas grade I" is justified (Böcking and Sinagowitz 1980; Mostofi 1980; Müller et al. 1980).

Adenocarcinomas with a labelling index of more than 0.5% should be included in the group of poorly differentiated adenocarcinomas because of their moderate nuclear anaplasia although histologically they seem to be well differentiated (see also Mostofi WHO 1980).

Prostatic carcinomas with a labelling index between 1.4 and 2.3% although these showed partially tubular structures were cytologically indistinguishable from undifferentiated and cribriform carcinomas. These are the least differentiated adenocarcinomas.

If the results of the present study are applied in the routine diagnostic of prostate carcinomas it becomes evident that some adenocarcinomas histologically diagnosed as well differentiated are actually "poorly differentiated" (Helpap and Otten 1981).

Furthermore, combined histological and cytological grading on transrectal or transurethral biopsies of the prostate may be helpful in differential diagnosis of prostatic carcinomas with uniform and pluriform structures (Kastendieck 1980). The existence of "differentiated" adenocarcinomas with cytological variables corresponding to labelling indices of more than 0.5% (moderate or marked nuclear anaplasia) implies that poorly differentiated or even undifferentiated parts of the carcinoma will be found in the remaining parts of the prostate and that the carcinoma may therefore be of pluriform structure.

The purpose of the present paper was to find a correlation between fixed

cell kinetic variables and the cytological characteristics of prostate cancer which can be applied to the data given in the literature (Gleason 1966; Esposti 1971; Rabes and Faul 1973; Mostofi 1975, 1980; Epstein and Fatti 1976; Faul et al. 1978; Voeth et al. 1978; Kastendieck 1977, 1980; Böcking and Sinagowitz 1980). On this cell kinetic analysis the histological and cytological grading may be helpful in the exact typing of well differentiated adenocarcinomas with a uniform structure, and in the exact classification of the total tumour mass in biopsy material.

The valuable assistance of Mrs. Inge Heim and of Mr. cand. med. Otten ist gratefully acknowledged.

References

Alken CE, Dhom G, Straube W, Braun JS, Kopper B, Rehker H (1975) Therapie des Prostata-Carcinoms und Verlaufskontrolle (III). Urologe A 14:112–116

Alken CE, Dhom G, Kopper B, Rehker H, Dietz R, Kopp S, Ziegler M (1977) Verlaufskontrolle nach Hochvolttherapie des Prostatacarcinoms. Urologe A 16:272–278

Böcking A, Sinagowitz E, Thon W, Nattenmüller E, Wagner J, Sandritter W (1979) Histologisches Grading des Prostatakarzinoms. Med Welt 30:275–279

Böcking A, Sinagowitz E (1980) Histologic grading of prostatic carcinoma. Path Res Pract 168:115–125

Dhom G (1977) Classification and grading of prostatic carcinoma. In: Grundmann E, Vahlensieck W (eds) Tumors of the male genital system. Recent Results in Cancer Research, Vol. 60, Springer, Berlin Heidelberg New York, pp 14–26

Dhom G (1978) Aktuelle Probleme der Epidemiologie und Pathologie des Prostatacarcinoms. Verh Dtsch Krebsges 1:193–203

Dhom G, Hautumm B (1975) Die Morphologie des klinischen Stadiums O des Prostatacarcinoms (incidental carcinoma). Urologe A 14:105–111

Epstein NA (1976) Prostatic carcinoma. Correlation of histologic features of prognostic value with cytomorphology. Cancer 38:2071–2077

Epstein NA (1976) Prostatic biopsy. A morphologic correlation of aspiration cytology with needle biopsy histology. Cancer 38:2078–2087

Epstein NA, Fatti LP (1976) Prostatic carcinoma. Some morphological features affecting prognosis. Cancer 37:2455–2465

Esposti PL (1971) Cytologic malignancy grading of prostatic carcinoma by transrectal aspiration biopsy. A five-year follow-up study of 469 hormone-treated patients. Scand J Urol Nephrol 5:199–209

Faul P, Schmiedt E, Kern R (1978) Die prognostische Bedeutung des zytologischen Differenzierungsgrades beim östrogenbehandelten Prostata-Carcinom. Verlaufskontrolle 496 östrogenbehandelter Prostata-Carcinom-Kranker bis zu einem Zeitraum von 5 Jahren. Urologe A 17:377–381

Gleason DF (1966) Classification of prostatic carcinomas. Cancer Chemother Rep 50:125–128

Gleason DF, Mellinger GT and the Veterans administration cooperative urological research group (1974) Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 111:58–63

Helpap B, Otten J (1981) Histologisch-cytologisches Grading von uniformen und pluriformen Prostatacarcinomen. Pathologe (in press)

Helpap B, Stiens R, Brühl P (1974) Autoradiographische Untersuchungen an inkubierten Prostatapunktaten nach Doppelmarkierung mit ^{14}C und ^{3}H-Thymidin. Beitr Pathol 151:65–74

Helpap B, Stiens R, Brühl P (1976) The proliferative pattern of the prostatic carcinoma before and under hormonal treatment. Z Krebsforsch 87:311–320

Kastendieck H (1977) Ultrastrukturpathologie der menschlichen Prostatadrüse. Veröffentlichungen aus der Pathologie 106. Fischer, Stuttgart New York

Kastendieck H (1980) Morphologie des Prostatacarcinoms in Stanzbiopsien und totalen Prostatektomien. Untersuchungen zur Frage der Relevanz bioptischer Befundaussagen. Pathologe 2:31–43

Leistenschneider W, Nagel R (1980) Zytologisches Regressions-Grading und seine prognostische Bedeutung beim konservativ behandelten Prostatakarzinom. Aktuelle Urol 11:263–275
Mellinger GT, Gleason D, Bailar J (1967) The histology and prognosis of prostatic cancer. J Urol 97:331–337
Mostofi FK (1975) Grading of prostatic carcinoma. Cancer Chemother Rep 59:111–117
Mostofi FK (1980) Histological typing of prostate tumours. International histological classification of tumours 22. World Health Organisation, Geneva
Müller HA, Altenähr E, Böcking A, Dhom G, Faul P, Göttinger H, Helpap B, Hohbach Ch, Kastendieck H, Leistenschneider W (1980) Über Klassifikation und Grading des Prostatacarcinoms. Verh Dtsch Ges Pathol 64:609–611
Murphy GP, Whitmore WF (1979) A report of the workshops of the current status of the histologic grading of prostate cancer. Cancer 44:1490–1494
Rabes H, Faul P (1973) Bestimmung der Proliferationsaktivität von menschlichen Prostata-Punktaten durch Thymidin-3H-Autoradiographie. Verh Dtsch Ges Pathol 57:318–322
Schröder FH, Mostofi FK, Belt E (1978) Malignitätsgrad und Prognose des Prostata-Carcinoms. Verh Dtsch Krebsges 1:255–256
Schubert GE, Ziegler H, Völter D (1973) Vergleichende histologische und zytologische Untersuchungen der Prostata unter besonderer Berücksichtigung östrogeninduzierter Veränderungen. Verh Dtsch Ges Pathol 57:315–318
Voeth Ch, Droese M, Steuer G (1978) Erfahrungen mit dem zytologischen Grading beim Prostatakarzinom. Urologe A 17:367–370

Accepted July 22, 1981