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Treatment of Prostatic Cancer With LH-RH Analogues

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Twenty-one of 32 patients with locally advanced prostatic cancer (stage C) were treated with the LH-RH analogue Buserelin for 7–19 months. After an initial sequence of subcutaneous injections, treatment was continued with intranasal spray application (three daily doses of 400 μg each) which ensured maintenance of serum testosterone within the range seen in castrated men.

To evaluate the response of the primary tumor to Buserelin, cytological regression was established for all patients by fine-needle aspiration biopsy every 3 months. The cytological results corresponded with those of DNA analyses of single-cell cytophotometry showing a statistically significant drop of the grade of aneuploidy or polyploidy when the prostatic carcinoma responded positively to Buserelin therapy.

Seventeen of 21 patients treated with the potent LH-RH analogue showed good therapy response. Four patients with no cytological signs of tumor regression received secondary treatment with estramustin phosphate because of hormone resistence. One patient had to be crossed over to cyclophosphamide, the third drug, for clinical progression after 15 months. Essential side effects have not been observed.

Continuous treatment of locally advanced prostatic cancer with Buserelin, combined with close control of the patient, offers not only a real alternative to surgical castration—as the patient is spared the psychical stress of orchiectomy—but also to estrogen therapy with its risk of cardiovascular side effects.

Key words: locally advanced prostatic cancer, conservative treatment, LH-RH analogues, therapy control, cytology

INTRODUCTION

Since Huggins and Hodges [1] discovered in 1941 that suppression or withdrawal of androgen slows down the growth of prostatic carcinomas for some time, this kind of *primary therapy* of the locally advanced, inoperable prostatic carcinoma with or without metastases has lost none of its validity. Androgen withdrawal by castration, however, is not accepted by all patients, and indirect suppression of

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androgen production by substances imitating the effect of estrogens may produce cardiovascular side effects in some patients, depending on dosage [2].

The isolation, characterization, and synthesis of the endogenous decapeptide LH-RH by Schally [3] in 1971 seemed to open new approaches to the treatment of endocrine fertility disturbances [4–9]. However, neither natural LH-RH nor the newly developed synthetic LH-RH analogue, which is more potent and produces a more persistent effect than the former, gave rise to great hopes. Difficulties with this therapy arise from the possible dose and time-dependent paradoxical antifertility effect of the substance demonstrated in various species [10–14].

Continuous injection of high doses of the potent LH-RH analogue Buserelin (D-Ser-(TBU)^{6}-EA^{10}-LH-RH; HOE 766) in rats resulted in a reduction of serum testosterone levels and of ventral prostate, seminal vesicle, and testis weight [12,13]. In men, too, prolonged administration of LH-RH analogues induced inhibition of testosterone synthesis, though incomplete, which offered new possibilities in the treatment of androgen-dependent conditions. In 1981, Redding and Schally [11] succeeded in inhibiting prostatic cancer growth by prolonged administration of potent LH-RH analogues in small-scale experiments with rats. This effect suggested employment of LH-RH analogues in the primary treatment of locally advanced or metastasing cancer in man as well.

PATIENTS AND METHODS

In the Department of Urology at the Free University of Berlin, Charlottenburg Medical Center, 32 patients with locally advanced prostatic carcinoma were treated with the LH-RH analogue Buserelin from June 1, 1981, to December 31, 1982. Thirty patients had stage C (T3, N 0/+, M 0) carcinoma, and two patients had stage D (T3, N 0/+, M 1) carcinoma with bone metastases. Twenty-one of these 32 patients have now been treated for 7–19 months.

Only these 21 patients, all with stage C carcinoma, will be reported on as they have been treated for longer than 6 months, and therapy response of locally advanced cancer *without* bone metastases or other clinical symptoms can be established solely by the regressive changes in the tumor itself [15–20], and that by histology, cytology, or DNA cytophotometry.

With orchiectomy, DES, or antiandrogen therapy, *definitive and accurate* evaluation of therapy response is possible *after 6 months*. If *no* regressive changes are manifest in the tumor itself at this point and therapy is continued, tumor progression or metastasis is likely to occur in the near future [17–19].

As our institution has been the first in West Germany to use Buserelin in the treatment of prostatic cancer, from June 1 to October 1981 various dosages and medication intervals were first investigated with nine patients in order to find the appropriate dose regimen for rapid and sustained reduction of serum testosterone to the level seen in castrated men [21].

As shown in Table I, Buserelin was given subcutaneously in two or three daily doses for periods varying from 3 to 14 days. Thereafter therapy was usually continued with three intranasal doses daily of 200 μg each. After three weeks, all dose regimens except one produced a fall of serum testosterone to levels found in castrated men, and maintained this "castrate" level for months. As for the exception, after a 2-week

TABLE I. Initial and Current Dose Regimen

Buserelin	Initial Dose Regimen
6/1/1981 – 30/10/1981	n = 9
n	**Dosage**
3	2 • 1000 mcg/s.c./day 1– 3 → 3 • 200 mcg/day/i.n.
2	3 • 1000 mcg/s.c./day 1– 6 → 3 • 200 mcg/day/i.n.
4	2 • 100 mcg/s.c./day 1–14 → **2 • 200 mcg/day/i.n.** or 3 • 200 mcg/day/i.n.
	Current dose regimen
	3 • 500 mcg/s.c./day 1– 7 → 3 • 400 mcg/day/i.n.

sequence of subcutaneous injections of 200 μg daily, the intranasal application of 200 μg twice daily proved insufficient for maintaining serum testosterone levels within this range (Table I).

Based on an agreement among several medical centers, the following standard dose regimen was adopted in mid-1982: subcutaneous injection of three daily doses of 500 μg for 7 days, followed by long-term intranasal application of three daily doses of 400 μg each.

Hormone Analyses

During the first 2 weeks of therapy, blood samples were taken twice daily at 8 a.m. and 4 p.m., and thereafter once weekly at 8 a.m., in order to determine serum levels of testosterone, LH, prolactin, and FSH by radioimmunoassay. In addition to the usual blood tests, serum thyroxin, T3, and cortisol were determined every 4 weeks.

The normal serum testosterone levels, as established by radioimmunoassay, range from 2.5 to 10 ng/ml in sexually mature men.

Testosterone levels in a control group of 40 surgically castrated men with prostatic cancer, aged 60 to 80 years, ranged from 0.1 to 0.8 ng/ml (mean value 0.28 ng/ml).

Clinical Follow-up Examinations

All patients underwent the examinations listed in Table II at intervals of 4 weeks, 3 months, and 6 months. As 30 of 32 patients had a nonmetastasing, locally advanced prostatic carcinoma without clinical symptoms (stage C; T3, N 0/+, M 0), the establishment of the cytological regression grade by aspiration biopsy and of the DNA content of tumorous nuclei by DNA cytophotometry were most important for the evaluation of tumor response to Buserelin.

TABLE II. Follow-up Examinations During Buserelin Therapy

Follow-up Examinations during Buserelin Therapy		
every 4 weeks	every 3 months	every 6 months
Blood Chemistry	Bone Scan	IVP
Rectal Palpation	Rectal Ultrasound	Cat Scan
Weight	Chest Film	
Physical Status	**Aspiration Biopsy** **DNA Cytophotometry**	Punch Biopsy

RESULTS

Hormone Studies

Prior to long-term treatment, the serum levels of testosterone and of the gonadotropic substances LH and FSH of all patients were found to be within standard range.

After 3 weeks of Buserelin treatment with a sequence of subcutaneous injections of 2 daily doses of 1,000 μg, three daily doses of 1,000 μg, and two daily doses of 100 μg each, followed by intranasal application of three daily doses of 200 μg, serum LH levels fell to lower than normal (below 5 mIU/ml) (Fig. 1), and serum testosterone decreased to less than 0.8 ng/ml (Fig. 2). These levels were reliably maintained, without exception, in all 21 patients over observation periods of 7–19 months.

Two patients received intravenous infusions of 1,500 μg Buserelin daily for 3 days, as it had been suggested that continuous infusion of high doses might bring about an accelerated fall of serum testosterone levels. Initial infusion and continuation of therapy with intranasal application of three daily doses of 400 μg Buserelin proved to have no such advantage over other modes of medication as the definite fall of serum testosterone to levels similar to those of castrated men only occurred after 3 weeks, as with other modes of medication. It therefore is of no clinical importance (Fig. 3).

Cytology

The rating of tumor response to Buserelin was based on the cytological regression grade found on fine-needle aspiration biopsy, and on the changes of DNA content in the tumorous nuclei measured by single-cell cytophotometry. As the patients were in stage C and had neither bone metastases nor clinical symptoms, this was the only parameter with which to objectify the effect of Buserelin on the primary tumor.

Occasional punch biopsies showed exactly the same results with regard to tumor regression grades.

Cytological regression grading defines the grade of regression in the primary tumor induced by any form of therapy.

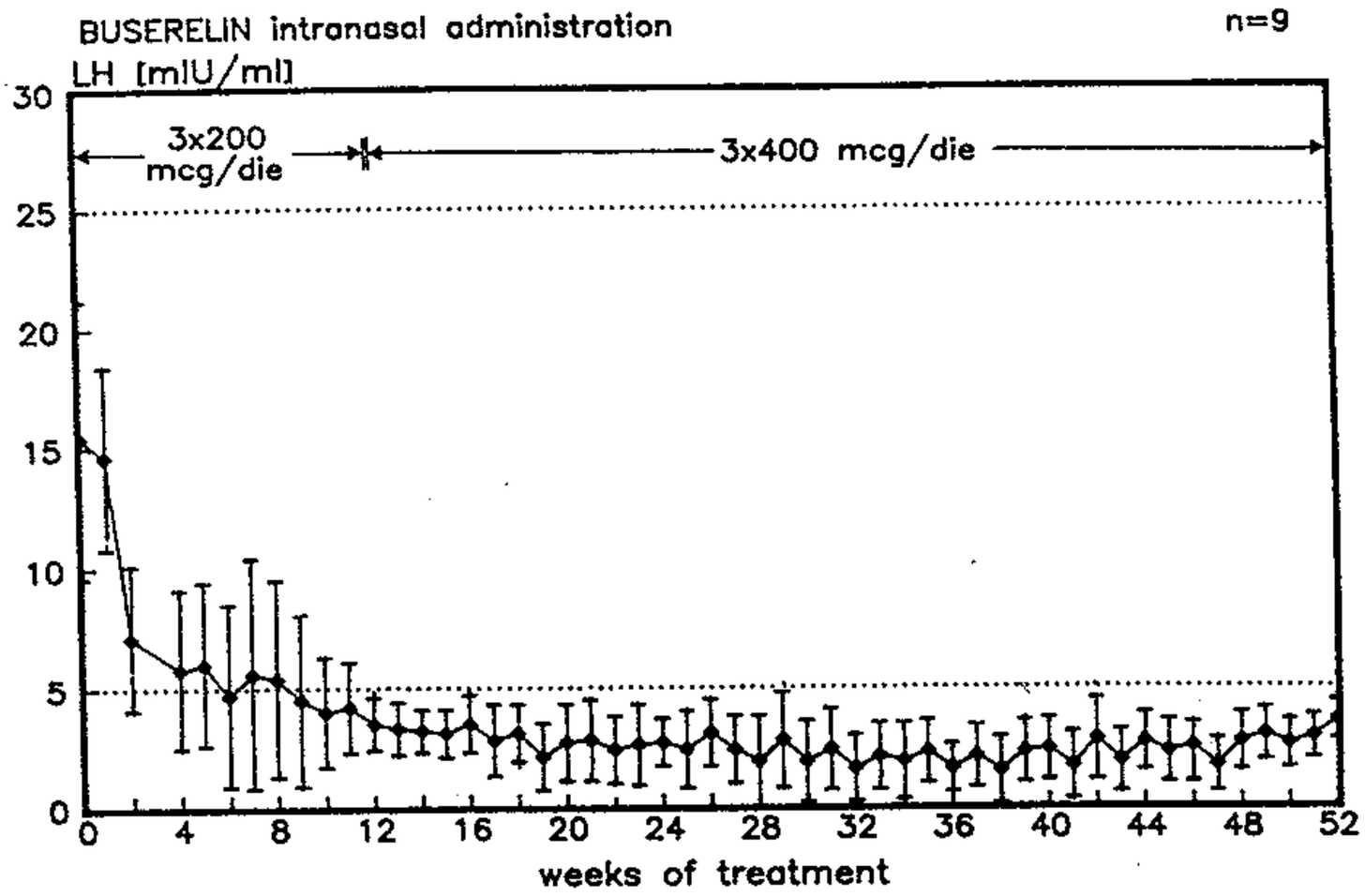


Fig. 1. Serum LH concentration after 3 weeks of Buserelin therapy. Typical initial LH increase before fall to levels lower than normal.

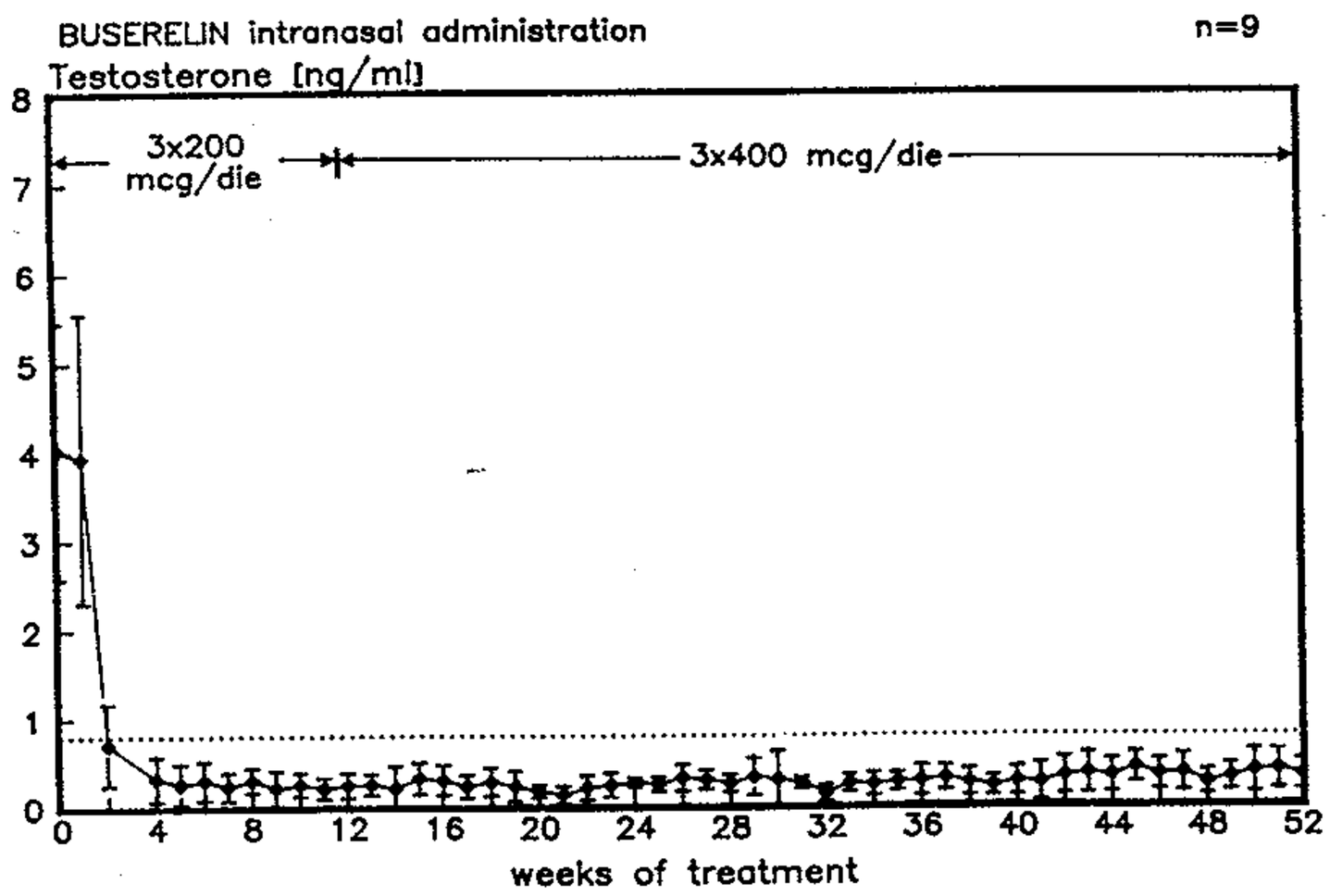


Fig. 2. Standard curve for serum testosterone of nine patients treated for maximally 12 months. After reduction of serum testosterone to "castrate" levels within 3 weeks reached with all dose regimens, maintenance of these levels (below 0.8 ng/ml), within standard variation, by intranasal spray application (three daily doses of 400 μg).

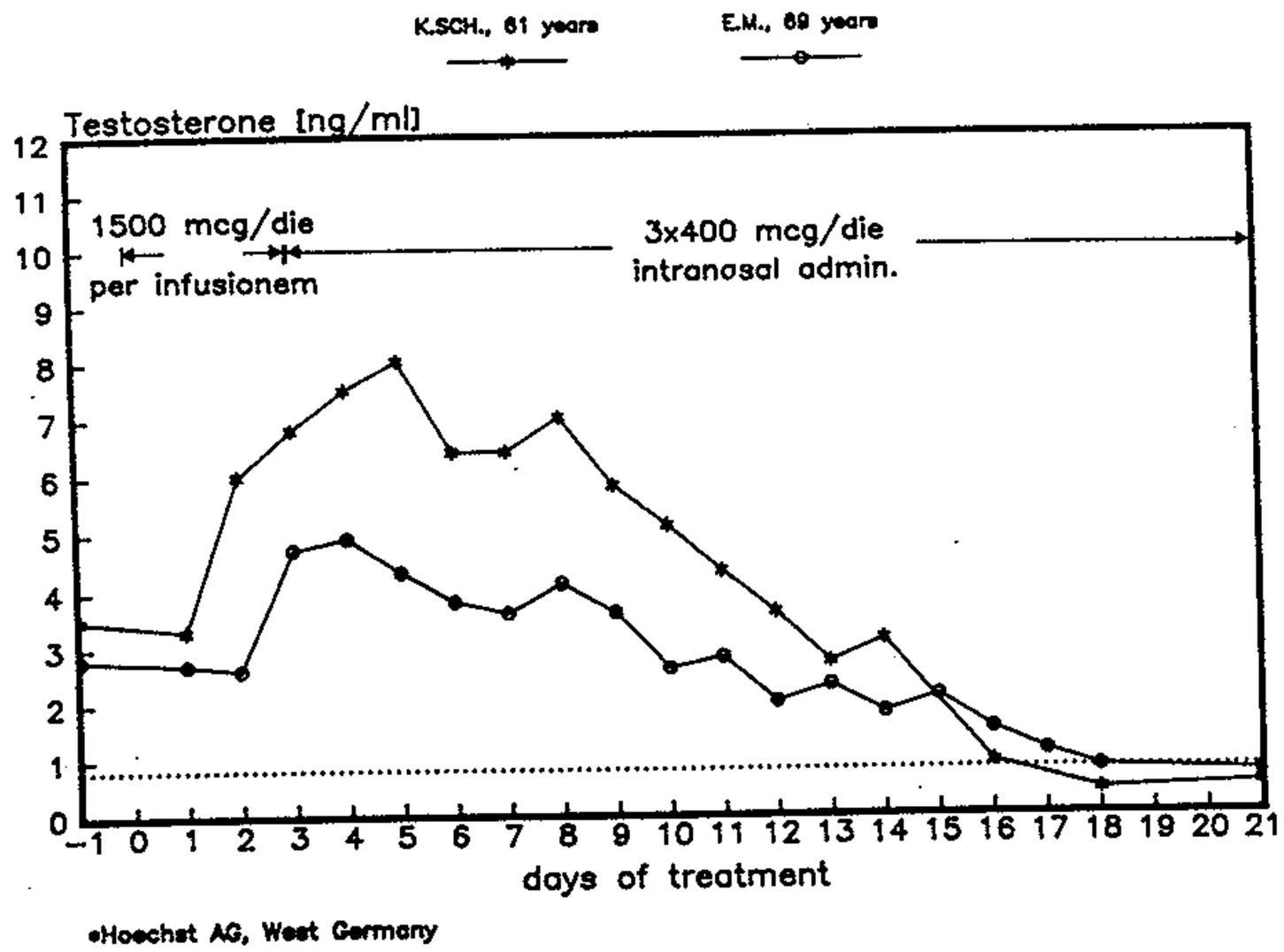


Fig. 3. Serum testosterone levels of two patients after continuous infusion round the clock (3 days, 1,500 μg daily). Continuation of therapy by intranasal application of three daily doses of 400 μg. No accelerated fall of serum testosterone after continuous infusion of high doses.

TABLE III. Grade of Regression According to Cytomorphological Criteria

Grade of regression	Effect of therapy	Cytomorphological criteria
0	very good	no carcinoma, marked regressive changes
II	good	epithelial atypias, carcinoma?, marked regressive changes
IV	satisfactory	few and small carcinoma cell clusters ("Residual carcinoma"). Marked regressive changes
VI	sufficient	carcinoma with marked regressive changes
VIII	poor	carcinoma with poor regressive changes
X	none	carcinoma without regressive changes

The most important signs of regression are found in the nucleus (pyknosis, decrease in size, rarefaction, etc) and in the nucleoli (decrease in size, disappearance, etc) [18,19,22].

The cytological criteria for regression correspond with those stated by Alken et al in 1975 [23] for regression grading by *histological examination*. The cytological regression grading as adopted by the Uro-Pathological Group in West Germany distinguishes six grades (Table III): Regression grades II–VI indicate good to sufficient therapy response of the tumor, established by the typical cytological changes. These regression grades do *not* require any change of therapy. Regression grades VIII and X indicate poor or no therapy response of the tumor.

Based on our experience with 300 patients and more than 1,000 control aspiration biopsies under six different types of therapy, we can state that regression grades VIII or X signal that tumor progression (metastases) is likely to occur within few months if therapy is not changed. The differences between good (grades II–VI) and poor regression (grade VIII or X) are statistically significant [18,19].

Intrapersonal reproducibility amounts to about 90%, interpersonal reproducibility to 80–85% [19].

Figure 4a and b shows, as an example, the cytological findings in a case of *good response* after 12 months of Buserelin therapy which is confirmed by the DNA cytophotogram (Fig. 6). In contrast to this, Figure 5a and b shows the results in a case of *almost nonexistent* therapy response. Therefore it was necessary to cross over to secondary EMCYT (estramustin phosphate) therapy after 7 months. After another 6 months local tumor progression occurred together with tumor breakthrough into the urinary bladder so that therapy had to be changed to a third drug, cyclophosphamide. The poor response to primary and secondary therapy is especially striking in the DNA cytophotogram (Fig. 8).

DNA Single Cell–Scanning Cytophotometry

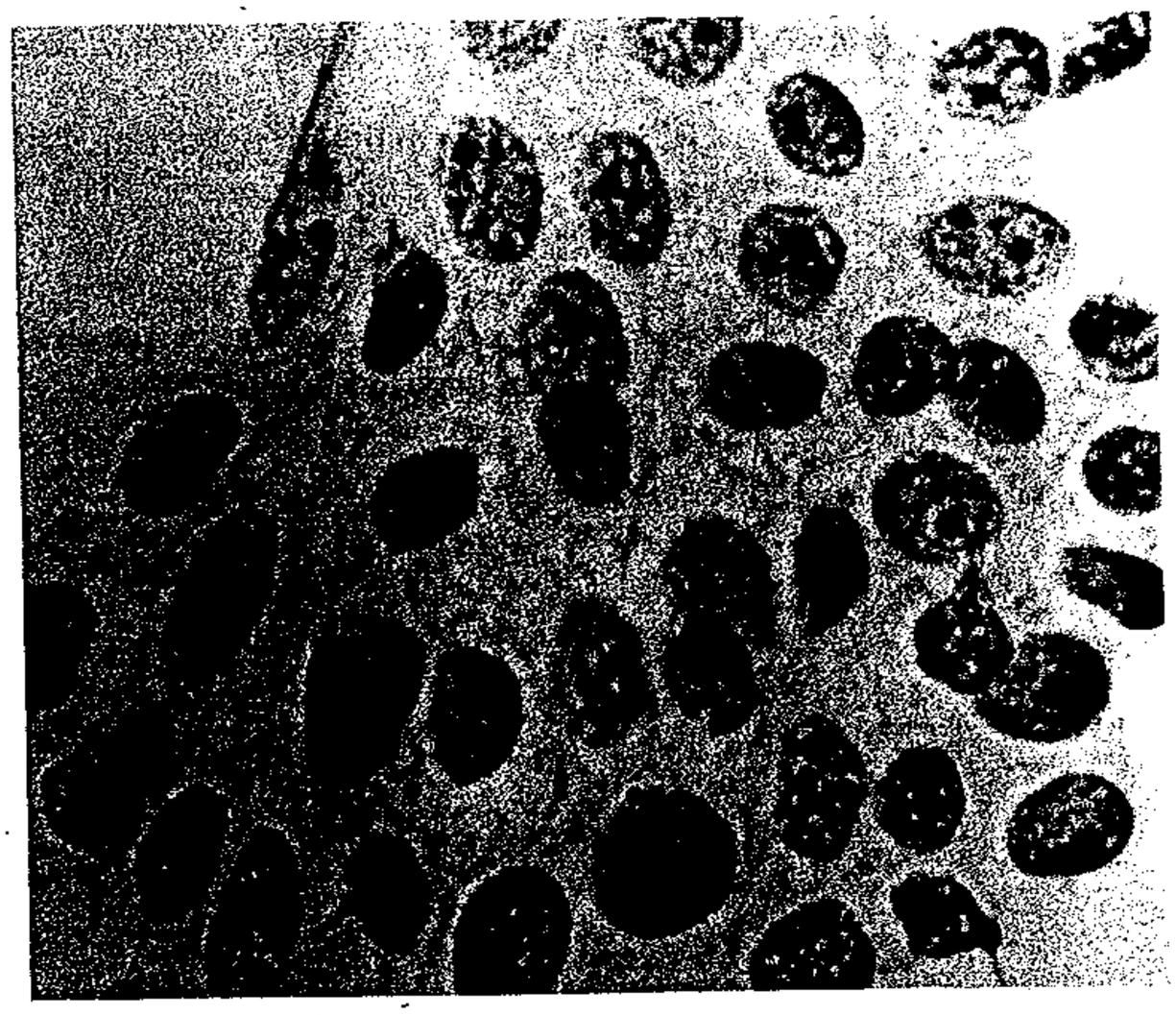
While cytology gives a morphological—and that is a qualitative—rating of therapy response, the analysis of the nucleus through single-cell cytophotometry affords a quantitative rating of regression in the primary tumor. Both methods, *particularly DNA cytophotometry*, can contribute greatly to a better understanding of biological tumor activity.

It has been known for years that tumor cell populations, corresponding to a change in the number of chromosomes toward aneuploidy, show a higher DNA content than normal tissues. This finding has been confirmed over and again by examinations of different organs, especially the mammas, lungs, and urinary bladder.

These results can easily be applied to the carcinoma of the prostate whose DNA content of the tumorous nuclei differs significantly from that of the adenoma, as was shown for the first time by Sprenger and co-workers in 1975 [24], later by Zetterberg [25], and also by us [16,17].

As far as we know we have been the first to investigate *sequentially* the response of the DNA content of tumorous nuclei in prostatic carcinomas to various therapeutical measures [16,17].

We found that a statistically significant drop of the grade of ploidy from aneuploid toward diploid occurs in the prostatic carcinoma when therapy is successful. If an aneuploidy remains unchanged during therapy, a negative clinical course of the disease may be predicted. Furthermore, we have been able to show that therapy-



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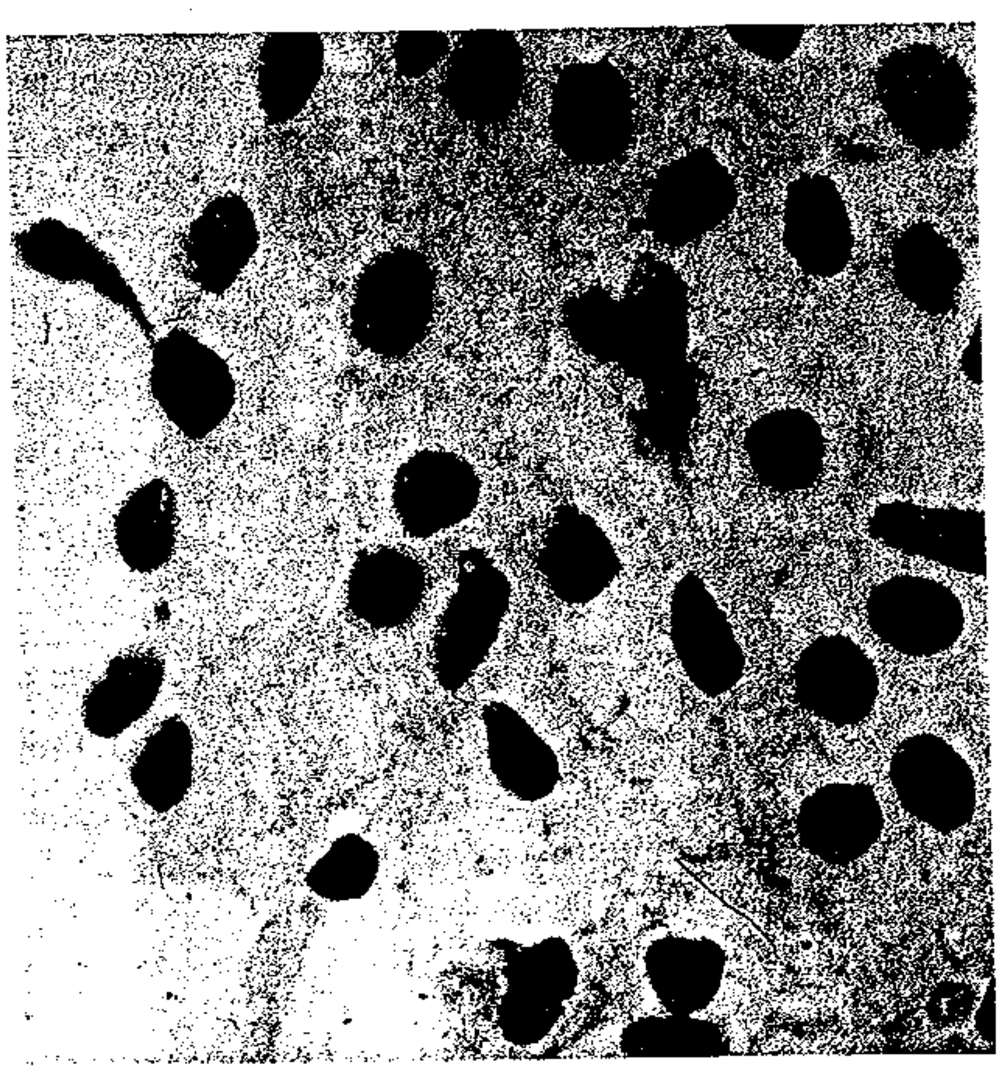

b

Fig. 4. a) K.P., 75 years, carcinoma stage C, grade II. Aspiration cytology *prior to* therapy. Nuclear polymorphy and hyperchromasy, irregularly displaced nuclei; some prominent nucleoli, partly no longer round; some nuclei have two nucleoli. Papanicolaou, magnification ×630. b) Same patient. Control cytology after 12 months of Buserelin therapy. Distinctly smaller nucleoles, in part disappearance. Dense vacuolation of nuclear chromatin. Nuclear pyknoses. Regression grade IV. Good therapy response. Papanicolaou, magnification ×630.

Fig. 5. a) E.H., 77 years, carcinoma stage C, grade II. Primary cytology prior to Buserelin therapy. Highly polymorphous and hyperchromatic nuclei. Nucleoli prominent and mostly no longer round, several nucleoli in one nucleus. Irregularly displaced nuclei. Papanicolaou, magnification ×630. b) Same patient, after 6 months of Buserelin therapy. Still highly polymorphous and hyperchromatic nuclei. Numerous prominent nucleoles which are no longer round. Often more than two nucleoli in one nucleus. No regressive changes as compared with primary cytology. Regression grade X. No therapy response. Secondary EMCYT therapy (see Fig. 8). Papanicolaou, magnification ×630.

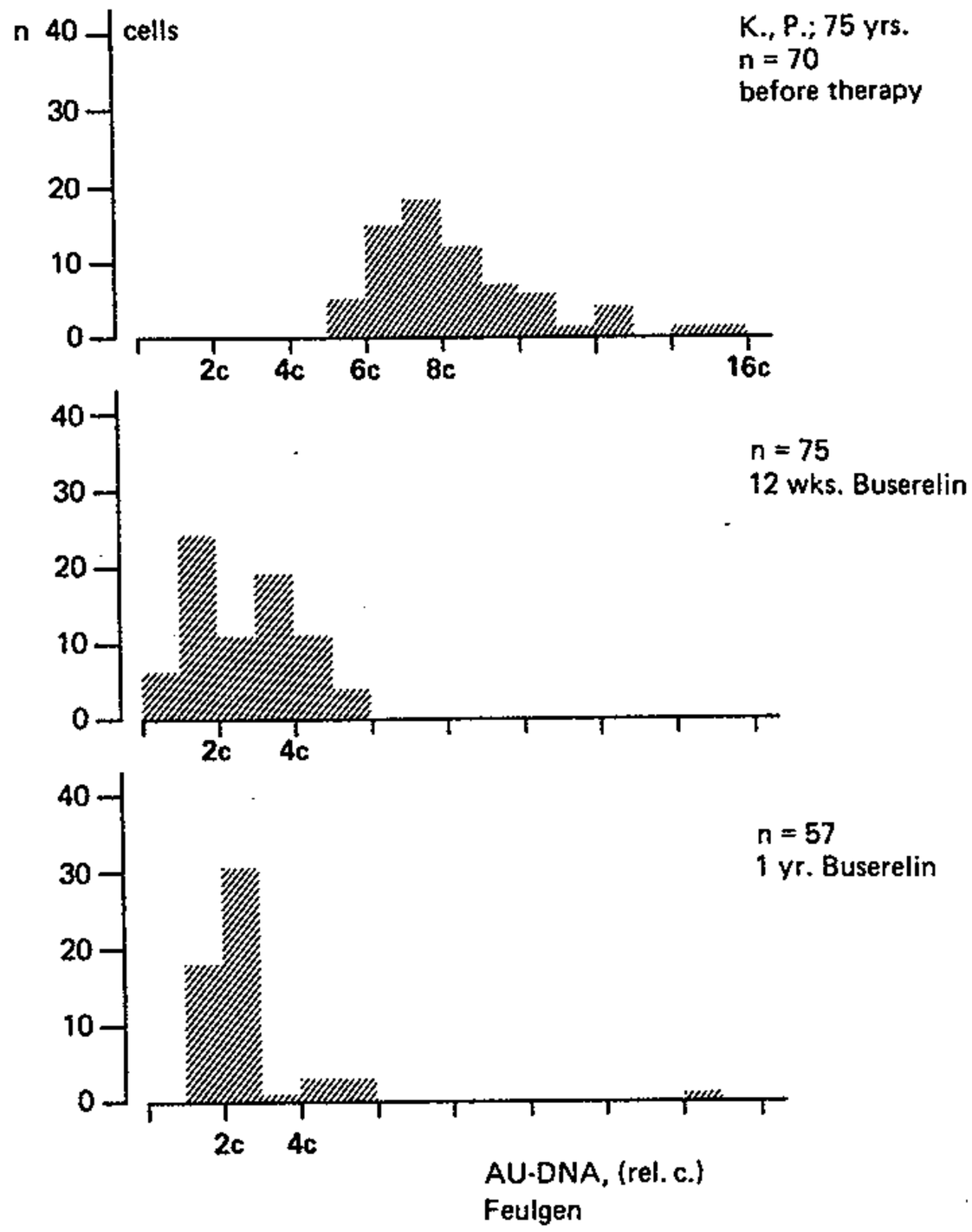

Fig. 6. DNA cytophotograms, K.P., 75 years, carcinoma grade II, prior to, after 12 weeks, and after 1 year of Buserelin therapy. Good response to Buserelin (see text and Fig. 4a, b).

resistant carcinomas of the prostate differ in their nuclear DNA content significantly from carcinomas with positive therapy response.

It is obvious from the studies conducted so far that nuclear DNA cytophotometry, though it is time-consuming as 60–80 individual tumorous nuclei have to be per patient, is a further reliable and especially good parameter for establishing positive therapy response in the prostatic carcinoma, and thus for realizing a prognostically oriented therapy.

METHODS

The cytophotograms establish the deviation of malignant nuclei from the diploid (2c) set of chromosomes of normal cells. This means that for each patient the DNA content of *normal leukocytes* (standard = 2c) has to be measured first and then compared with that of the tumorous nuclei. The values obtained by this comparison are given on the abscissa, in so-called Arbitrary Units (AU), and the exact number (n) of nuclei measured each time appears on the ordinate (Figs. 6–8). Accordingly, 2c means diploid, 4c tetraploid, and 8c octaploid.

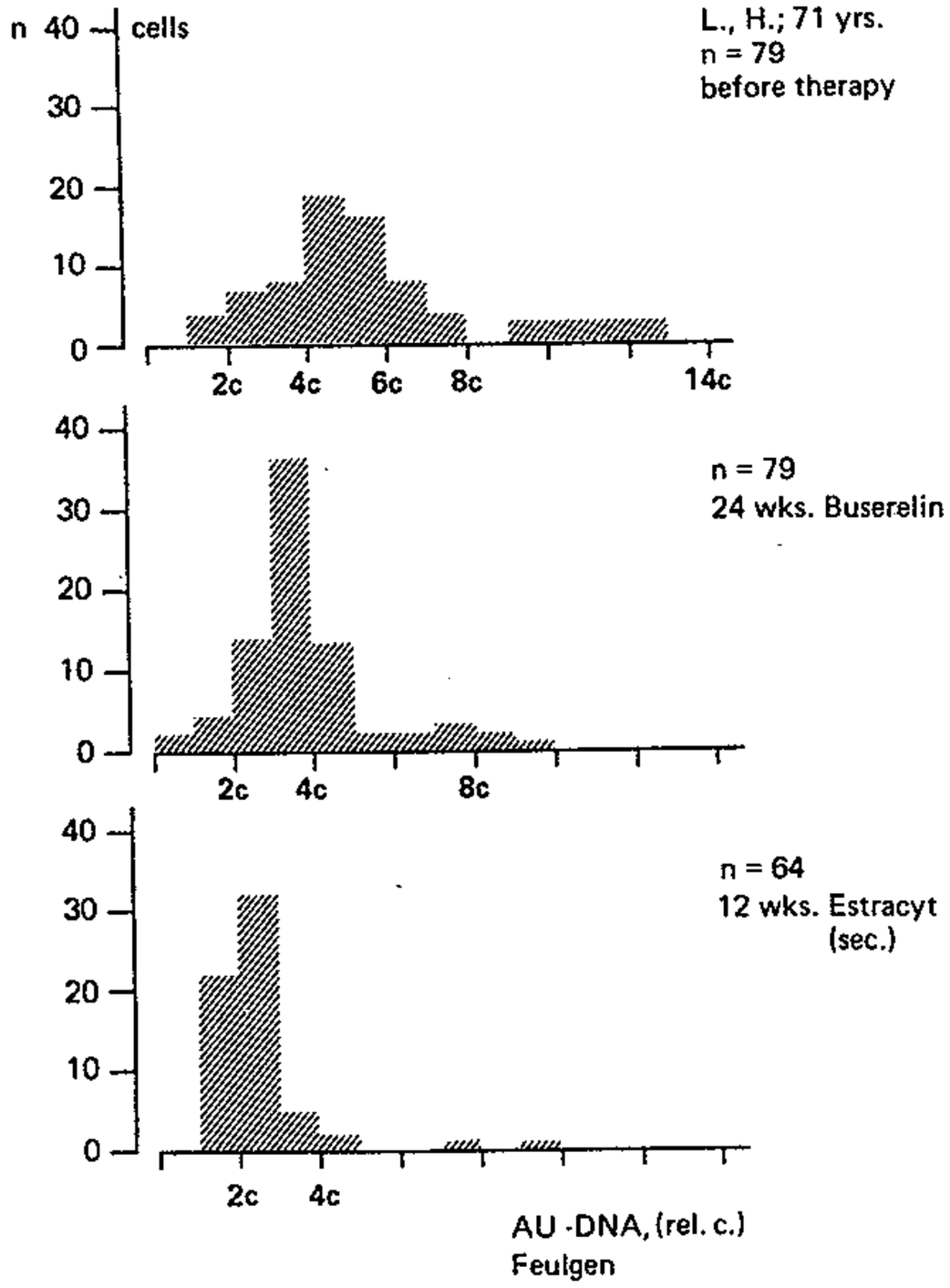

Fig. 7. DNA cytophotograms, L.H., 71 years, carcinoma grade II, prior to and after 24 weeks of Buserelin therapy. After 24 weeks, unsatisfactory therapy response. After 12 weeks of secondary EMCYT therapy, good response (see text).

Results of DNA Cytophotometry

The prognostic value of DNA cytophotograms for Buserelin therapy is the same as for any other therapy; it is statistically significant and can be reproduced in the sample at any time. Figures 6–8 show the cytophotograms of three patients with different therapy responses.

Figure 6: K.P., 75 years, grade II carcinoma. Prior to therapy there was a broad DNA peak in the 8c range with values spreading up to 16c. After 12 weeks of therapy a clear shift to the left, into the 4c and 2c range, is seen. This finding, after 12 weeks, promised good therapy response of the tumor which was verified by the cytophotogram taken after 1 year of Buserelin therapy, showing a slim peak in the 2c range and only very few values between 4c and 6c. The cytophotograms correspond with the findings on aspiration biopsy (see Fig. 4a, b) and the clinically stable condition of the patient after 15 months of therapy.

Figure 7: L.H., 71 years, grade II carcinoma. Prior to Buserelin therapy, the DNA frequency peak is between 4c and 6c, with some values spreading to 14c. After

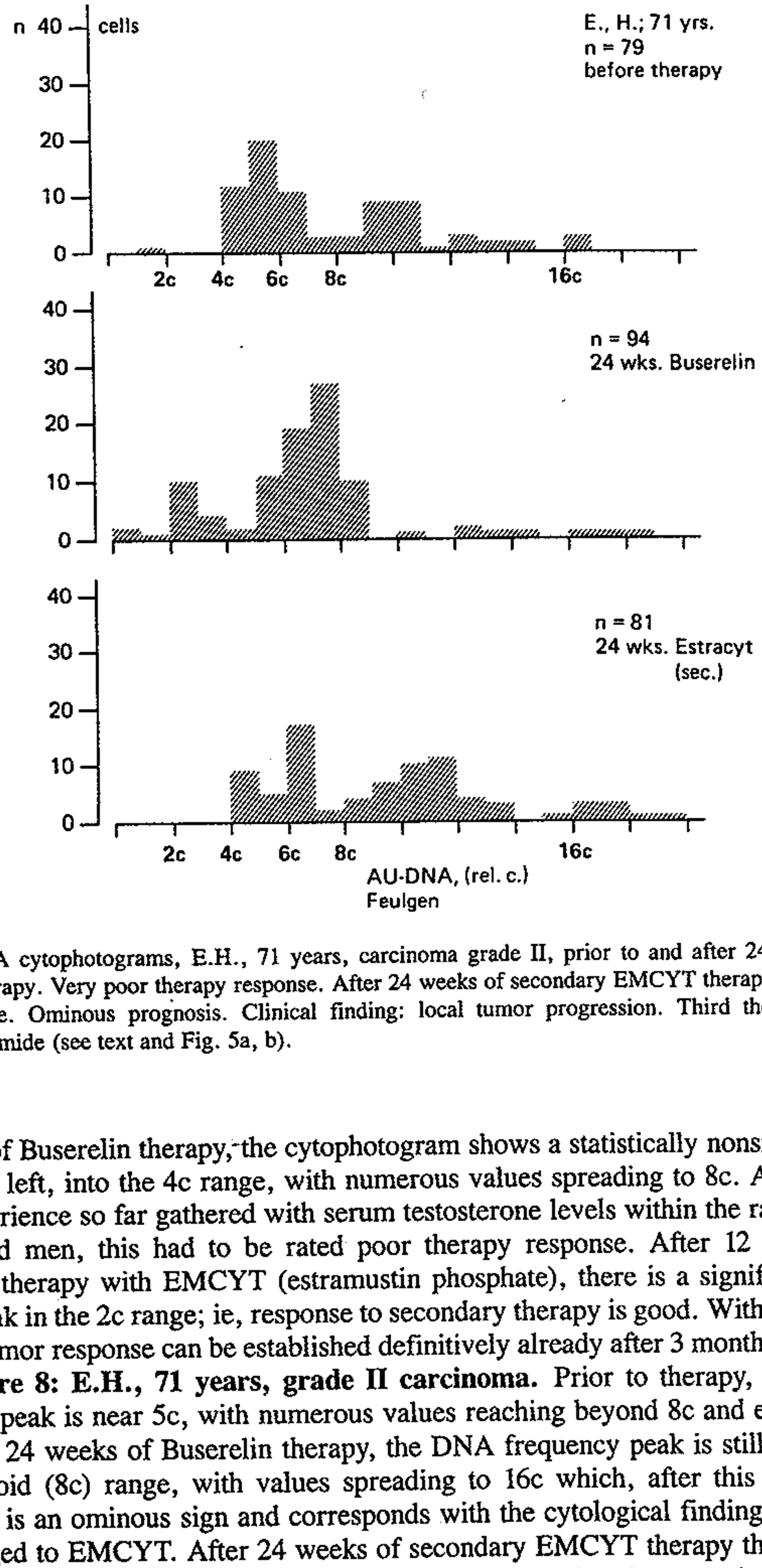

Fig. 8. DNA cytophotograms, E.H., 71 years, carcinoma grade II, prior to and after 24 weeks of Buserelin therapy. Very poor therapy response. After 24 weeks of secondary EMCYT therapy also very poor response. Ominous prognosis. Clinical finding: local tumor progression. Third therapy with cyclophosphamide (see text and Fig. 5a, b).

24 weeks of Buserelin therapy, the cytophotogram shows a statistically nonsignificant shift to the left, into the 4c range, with numerous values spreading to 8c. According to the experience so far gathered with serum testosterone levels within the range seen in castrated men, this had to be rated poor therapy response. After 12 weeks of secondary therapy with EMCYT (estramustin phosphate), there is a significant frequency peak in the 2c range; ie, response to secondary therapy is good. With EMCYT therapy, tumor response can be established definitively already after 3 months [16,17].

Figure 8: E.H., 71 years, grade II carcinoma. Prior to therapy, the DNA frequency peak is near 5c, with numerous values reaching beyond 8c and even up to 16c. After 24 weeks of Buserelin therapy, the DNA frequency peak is still far up in the octaploid (8c) range, with values spreading to 16c which, after this period of treatment, is an ominous sign and corresponds with the cytological finding. Therapy was changed to EMCYT. After 24 weeks of secondary EMCYT therapy there is still a marked aneuploidy of the tumor, with widely scattered values between 4c and 16c—

TABLE IV. Cytological and Clinical Response to Buserelin Therapy

Buserelin	**Response to Treatment**
Follow-up Examinations: **CYTOLOGY — DNA Cytophotometry**	(6–19 months)
Stage C (T3, NO/+/**MO**)	**n = 21**
Grade of Regression	n %
II – VI	17 (80,9)
VIII – X	4 (19,1) → 3 x EMCYT: 7, 7, 12 months → 1 x EMCYT: (sec.) 15 months → Cyclo-phosphamid

ie, resistance to secondary therapy as well. This finding was *clinically* verified by local tumor progression with breakthrough into the urinary bladder. Therapy was modified by crossover to the third drug, cyclophosphamide.

Cytological and Clinical Results

The clinical results of the 21 patients treated for 7–19 months are based mainly on cytological regression grades and DNA cytophotometry, and not so much on the clinical course of the disease. Table IV shows the response to Buserelin therapy according to cytological regression grades.

Seventeen of the 21 patients (80.9%) had good to sufficient regression grades (II to VI), whereas four patients showed only slight or no regression signs after 6–12 months of therapy—ie, regression grades VIII or X. These four patients were crossed over to *secondary* EMCYT therapy, in three cases after 7 months, and in one case after 12 months. In three cases, response to secondary therapy was good (Fig. 7). In one case the tumor did not respond to EMCYT either, and clinical progression set in accompanied by a breakthrough of the tumor into the urinary bladder (T4) so that therapy had to be changed to cyclophosphamide after 15 months. *At the time of crossover, the serum testosterone levels of all four patients were clearly within "castrate" range.*

This small but very thoroughly controlled series has confirmed the fact that about 20% of locally advanced prostatic carcinomas are not hormone-dependent; ie, the tumors do not respond to androgen withdrawal as demonstrated by the lack of regressive changes.

Side Effects

Two patients complained about itching in the nose, and four patients had hot flashes, all of which disappeared without specific therapy within 6–8 weeks.

All patients who had still been sexually active before therapy suffered complete loss of libido and potency.

DISCUSSION

The therapy of the locally advanced prostatic carcinoma with or without metastases in the nonsymptomatic stage still is a controversial matter, particularly concerning the question of when to start therapy [2,23,26]. Especially in the United States it is advocated not to start therapy before symptoms (bone pains, obstruction) occur [2]. In many European countries, however, immediate orchiectomy and/or estrogen therapy for stage C (T3) carcinomas still is common practice [23,26].

As the potent LH-RH analogue Buserelin leads to a reduction of serum testosterone to such levels as seen in castrated men and, with sufficient dosage, can also be maintained within this range over 12 months and longer, Buserelin offers itself for the treatment of locally advanced prostatic cancer to avoid orchiectomy and estrogen therapy with its negative side effects.

As has been demonstrated with so far 32 patients, serum testosterone can be definitively reduced to the levels found in castrated men, providing adequate dosage, and, in the case of 21 patients, these levels have been maintained for 7–19 months. For each patient control aspiration biopsies have been performed at intervals of 3–6 months, and DNA cytophotometry every 6 months. The therapy study with 30 patients in stage C who had neither metastases nor clinical symptoms (six of them even had grade III carcinoma) was judged justified since aspiration biopsy together with the cytological methods afford a unique means of examining the tumor itself, and, moreover, we have extensive experience with these methods (more than 300 patients/1,000 bioptic controls).

Only 21 out of 32 patients have been taken into this study. According to our experience with Buserelin therapy, the control criteria valid for orchiectomy or estrogen therapy also apply to Buserelin therapy, as in principle, Buserelin belongs to the same type—ie, it causes androgen reduction to values similar to those of castrated men. With this therapeutic principle, the regression grade, and thus therapy response, can be definitively established by cytology after 6 months, whereas EMCYT therapy permits regression grading as early as 3 months after initiating therapy [16,17].

We have been able to prove that rectal palpation results in false-positive diagnoses in at least 50% of cases, especially when therapy response is positive; ie, findings on palpation suggest that the tumor does not respond to therapy. For this reason aspiration biopsy is the only reliable parameter for therapy control [15,18].

The importance of regular bioptic control also lies in the fact that four of 21 patients did not respond to androgen withdrawal, and three of them showed good responses only after secondary EMCYT therapy.

One patient with primary grade II carcinoma—though his serum testosterone level remained constant within the range found in castrated men—entered into local progression accompanied by a breakthrough of the tumor into the urinary bladder even after secondary EMCYT therapy, so he now has to be treated with cyclophosphamide.

This series has again proved that the serum testosterone level is a reliable prognostic parameter, as known from the literature [27].

CONCLUSIONS

Our investigation of 21 patients treated for more than 7 months has proved the LH-RH analogue Buserelin to be potent enough to reduce serum testosterone to levels

similar to those seen in castrated men within 3 weeks, and maintain this level for 1 year and longer under continuous therapy with intranasal Buserelin application. Intranasal application is uncomplicated even for elderly patients, and compliance has so far been very good as shown by short-term serum testosterone controls. It is a decisive advantage of Buserelin in the treatment of locally advanced prostatic cancer that it offers an alternative to both orchiectomy with its psychical stress, and estrogen therapy with its negative side effects.

So far nothing is known about the duration of this drug-induced castration effect. Presumably it will not differ substantially from that of orchiectomy or estrogen therapy.

In the present stage of investigation, patients in clinical stage C (T3) should be controlled as to the effect of Buserelin on the primary tumor by aspiration or punch biopsy at least every 6 months, since four of 21 patients had a hormone resistance or clinical progression, despite the fact that their serum testosterone levels remained within the range found in castrated men.

REFERENCES

1. Huggins C, Stevens RE, Hodges CV: Studies on prostatic cancer: Effects of castration on advanced carcinoma of prostate gland. Arch Surg 43:209, 1941.
2. Veterans Administration Cooperative Urological Research Group: Treatment and survival of patients with cancer of the prostate. Surg Gynaecol Obstet 124:1011, 1967.
3. Schally AV, Arimura Y, Baba RMG, Nair H, Matsuo T, Redding W, Debeljuk L, White WF: Isolation and properties of the FSH- and LH-releasing hormone. Biochem Biophys Res Commun 43:393, 1971.
4. Bergquist C, Nillius SJ, Bergh T, Skarin G, Wide L: Inhibitory effects on gonadotropin secretion and gonadal function in men during chronic treatment with a potent stimulatory luteinizing hormone–releasing hormone analogue. Acta Endocrinol 91:601, 1979.
5. Cusan L, Auclair C, Belanger A, Ferland L, Kelly PA, Seguin C, Labrie F: Inhibitory effects of long-term treatment with a luteinizing hormone–releasing hormone agonist on the pituitary-gonadal axis in male and female rats. Endocrinology 104:1369, 1979.
6. Faure N, Lemay A, Belanger A, Labrie F: Inhibition of androgen biosynthesis in the human male by chronic administration of (D-Ser-TBU6-des-Gly-NH2-10)-LHRH-ethylamide (Buserelin). In Zatuchni GI, Shelton JD, Sciarra JJ (eds): "LHRH Peptides as Female and Male Contraceptives." Philadelphia: Harper and Row, 1981, p 307.
7. Hardt W, Schmidt-Gollwitzer M, Von der Ohe M, Nevinny-Stickel J: Der Einfluß der Dauermedikation des LH-RH-Analogons Buserelin auf die Zyklusfunktion. Geburts Frauenheilk 41:791, 1981.
8. Schmidt-Gollwitzer M, Hardt W, Schmidt-Gollwitzer K, Von der Ohe M, Nevinny-Stickel J: Influence of the LH-RH analogue buserelin on cyclic ovarian function and on endometrium. A new approach to fertility control? Contraception 23:187, 1981.
9. Wiegelmann W, Solbach HG, Kley HK, Krueskemper HL: LH- and FSH-response to long-term application of LH-RH analogue in normal males. Horm Metab Res 9:521, 1977.
10. Auclair C, Kelly PA, Coy DH, Schally AV, Labrie F: Potent inhibitory activity of D-Leu-des-GLY-NH2-10-LHRH ethylamide on LH, HCG and Prl testicular receptor levels in the rat. Endocrinology 101:1890, 1977.
11. Redding TW, Schally AV: Inhibition of prostate tumor growth in two rat models by chronic administration of D-Trp6-analogue of luteinizing hormone–releasing hormone agonists. Proc Natl Acad Sci USA 78:6509, 1981.
12. Rivier C, Rivier J, Vale W: Chronic effects of D-Trp6, Pro9-NEt-luteinizing hormone–releasing factor on reproductive processes in the male rat. Endocrinology 105:1191, 1979.
13. Sandow J, Von Rechenberg W, Jerzabek G, Engelbart K, Kuhl H, Fraser H: Hypothalamic-pituitary-testicular function in rats after supraphysiological doses of a highly active LHRH analogue (Buserelin). Acta Endocrinol 94:489, 1980.
14. Sandow J, Clayton RM, Kuhl H: Pharmacology of LH-RH and its analogues. In: "Endocrinology

of Human Infertility: New Aspects." Serono Symposium, Oxford, 1980. New York: Academic Press, 1981, p 221.
15. Leistenschneider W, Nagel R: Zytologisches Regressionsgrading und seine prognostische Bedeutung beim konservativ behandelten Prostatakarzinom. Akt Urol 11:263, 1980.
16. Leistenschneider W, Nagel R: Estracyt therapy of advanced prostatic cancer with special reference to control of therapy with cytology and DNA-cytophotometry. Eur Urol 6:111, 1980.
17. Leistenschneider W, Nagel R: Cytological and DNA-cytophotometry monitoring of the effects of therapy in conservatively treated prostatic carcinoma. Scand J Urol Nephrol Suppl 55:197, 1980.
18. Leistenschneider W, Nagel R: Zytologische Primärdiagnostik und Therapiekontrolle beim Prostatakarzinom. Extracta Urol 5:581, 1982.
19. Leistenschneider W, Nagel R: Zytologische Therapiekontrolle des konservativ behandelten Prostatakarzinoms. Klassifikation und klinische Bedeutung. Urologe A, 22:144, 1983.
20. Sewell RA, Braren V, Wilson SK, Rhamy RK: Extended biopsy follow up after full course radiation for resectable prostatic carcinoma. J Urol 113:371,1975.
21. Borgmann V, Hardt W, Schmidt-Gollwitzer M, Adenauer H, Nagel R: Sustained suppression of testosterone production by the LH-RH agonist Buserelin in patients with advanced carcinoma of the prostate—A new therapeutical approach? Lancet 1097, 1982.
22. Zetterberg A: Cytochemical methods of malignancy grading. WHO Collaborating Center Meeting on Prostate Cancer, March 12–15, 1979, Stockholm.
23. Alken CE, Dhom G, Straube W, Braun JS, Kopper B, Rehker H: Therapie des Prostatakarzinoms und Verlaufskontrolle III. Urologe A 14:112, 1975.
24. Sprenger E, Volk L, Michaelis WW: Die Aussagekraft der Zellkern-DNS-Bestimmung bei der Diagnostik des Prostatakarzinoms. Beitr Path 153:370, 1974.
25. Zetterberg A, Esposti PL: Cytophotometric DNA-analysis of aspirated cells from prostatic carcinoma. Acta Cytol 20:46, 1979.
26. Altwein JE: The historical contribution of European urology to scientific progress in the field of prostatic neoplasia. In Jacobi GH, Hohenfellner R (eds): "Prostate Cancer." Baltimore: Williams and Wilkins, 1982, p 227.
27. Scott WW, Menon M, Walsh PC: Hormonal therapy of prostatic cancer. Cancer 45:1929, 1980.