Urol Res (1992) 20:133–138

Urological Research

Partial purification and characterization of a growth factor from human hyperplastic prostatic tissues

A. Dignaß and A. W. Holldorf

Institute of Physiological Chemistry, Ruhr-University Bochum, Bochum, FRG

Accepted: September 1, 1991

Summary. A growth factor capable of stimulating DNA synthesis of Balb/c 3T3 cells was purified by heparin-Sepharose column chromatography about 1900-fold from the cytosol of human prostatic tissues obtained at autopsy or open prostatectomy. This growth factor bound to heparin-Sepharose in the presence of 0.5 mol/l NaCl and was eluted by 1.0–1.55 mol/l NaCl. Its molecular weight was estimated to be 68000 by SDS-polyacrylamide gel electrophoresis. The amino acid composition was determined and compared with the data of other growth factors, which revealed no striking conformity. Distribution of growth factor activity was investigated in mechanically separated prostatic tissues of benign prostatic hyperplasia. The separation scheme provided two fractions: the stromal fraction consisting mainly of fibroblasts, fibers and smooth muscle, and the epithelial fraction consisting of epithelial cells. The specific growth-stimulating activity in the stromal fraction was about 2-fold that in the epithelial fraction. Referred to the total activity of whole tissue, about 74% of the activity could be detected in the stromal fraction, while only about 5% was detectable in the epithelial fraction. This study demonstrates the existence of a growth factor in human benign hyperplastic prostatic tissues, showing a remarkable distribution of growth factor activity, which may play a role in the pathogenesis of benign prostatic hyperplasia.

Key words: Benign prostatic hyperplasia – Growth factor – Heparin-Sepharose chromatography

Although benign prostatic hyperplasia (BPH) is probably the most common neoplastic growth that occurs in men [31], the etiology remains unclear. Over 290000 surgical procedures are performed each year in the United States to relieve the symptoms arising from BPH. This makes BPH the second leading cause of surgery in men, with an overall medical cost well in excess of $1 billion per year in the United States [31]. Numerous reports on the origin and the development of BPH have appeared in the literature. All available information points to two leading factors in the development of BPH: the testes and aging [31, 32]. As long ago as 1940 Huggins and Stevens [10] suggested that at least prostatic epithelium is controlled by the testis. The role of the hormones, however, may be permissive rather than causal [29, 33]. A synergistic action of androgens and estrogens seems probable [30, 31, 33]. On the other hand, stromal-epithelial interactions may play a role in the pathogenesis of BPH [4, 31]. As early as 1939, Deming and Neumann [6] suggested that a growth-promoting substance produced by the prostate might be the cause of BPH. Their paper, however, did not stimulate the search for such a factor, and it was not until 1979 that Jacobs et al. [11] first reported a growth-promoting factor in crude extracts of human prostatic tissues. Meanwhile several investigators have been able to demonstrate and purify a number of different growth factors in human prostatic tissues [9, 12, 21, 26, 27, 29]. Nevertheless, the role of these growth factors remains unclear.

The facts described above emphasize the need for further investigation to find the etiology of this disorder and to provide alternative forms of treatment. In this study, the purification and further characterization of a growth factor from human hyperplastic prostatic tissues is presented.

Materials and methods

Assay for growth factor activity

Growth factor activity was determined by measuring the ability of samples to stimulate the incorporation of [^{3}H]thymidine into the DNA of quiescent Balb/c 3T3 cells (clone A 31, ATCC NO. CCL 163, Flow Laboratories GmbH, Meckenheim) as described elsewhere [13, 22, 27, 29], with modifications as described below. Balb/c 3T3 cells (6×10^4/ml) were plated in 96-well multiwell plates (Nunc, Wiesbaden, FRG) with 200 µl of Dulbecco's modified Eagle's medium (DMEM) containing 10% newborn calf serum (NCS) and 1% antibiotic solution (10.5 mg/ml streptomycine, 6.4 mg/ml penicillin G). DMEM and NCS were from Gibco Europe (Karlsruhe, FRG), antibiotics from Sigma Chemie (Taufkirchen, FRG). After

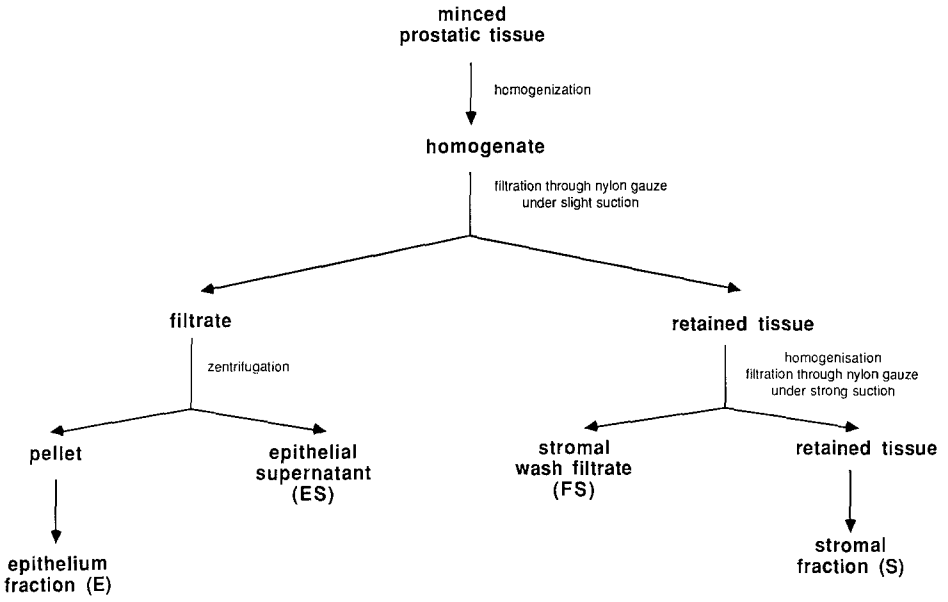

Fig. 1. Scheme of mechanical separation of human prostatic tissue [14]

incubation for 24 h at 37°C in a humidified atmosphere of 5% CO_2 in air the medium was replaced by 200 µl DMEM containing 1% NCS and 1% antibiotic solution. After incubation at 37°C for 48 h test samples and control material were added in volumes ranging from 10–30 µl/well. Immediately after addition of the test samples and control material, 10 µl of [^{3}H]thymidine (Amersham Buchler, Braunschweig, FRG) solution (104 µCi/ml; 6.7 Ci/mmol) was added to each well. After incubation for at least 24 h at 37°C the cells were washed once with 250 µl of phosphate-buffered saline (PBS) and incubated twice with 150 µl methanol per well at room temperature for 5 min each time. Then the cells were washed twice with 20 µl/well of distilled water and three times with 200 µl/well of cold 10% (w/v) trichloroacetic acid solution. DNA was solubilized with 200 µl/well of 0.5 mol/l NaOH and mixed with 3 ml Opti-Fluor scintillation fluid (Packard Instruments, Frankfurt, FRG). Radioactivity was counted with a Minaxi Tri-Carb 4000 Series Liquid Scintillation Counter (Packard Instruments, Frankfurt). All measurements were done at least in duplicate. A relative mitogenic activity (RMA) was calculated as follows [25–27]:

$$\text{RMA} = \frac{\text{CPM (test sample)} - \text{CPM (sample buffer)}}{\text{CPM (10\% NCS)} - \text{CPM (sample buffer)}}$$

One unit of activity was defined as a RMA equal to one, i.e. a response equal to that produced by 10% NCS.

Tissue homogenization and growth factor isolation

Human hyperplastic prostatic tissues were obtained at open prostatectomy or at autopsy. They were frozen and stored at −75°C for up to 3 months before use.

Finely minced prostatic tissue was homogenized in 10 volumes (v/w) of 10 mmol/l Tris-HCl (pH 7.5) with a Sorvall Omni mixer at a setting of 10 for 1 min and 1-min intervals with cooling (5 times). The homogenate was centrifuged at 38000 *g* for 1 h. The resulting supernatant was filtered through glass wool and used as the starting material (cytosol). Further purification was carried out with heparin-Sepharose column chromatograpy as described by Nishi et al. [21] with slight modifications. The cytosol of BPH tissue was supplemented with 2–4 mol/l NaCl to give a final NaCl concentration of 0.5 mol/l. The solution was applied to a heparin-Sepharose column (diameter 1.5 cm, bed volume about 9.5 cm^3) equilibrated with 10 mmol/l Tris-HCl (pH 7.5) containing 0.5 mol/l NaCl. The column was washed with the same buffer until the absorbance of the eluate reached baseline. The absorbed protein was eluated with a gradient (300 ml) of 0.5–3.0 mol/l NaCl. All procedures were carried out as close to 0°C as possible. NaCl concentrations in gradient fractions were determined by measuring the conductivity with a Conductivity Meter CDM 3 (Radiometer Copenhagen, Denmark). Growth factor activity of sample fractions was measured as described above.

Mechanical separation of prostatic tissue

All procedures were carried out as close to 0°C as possible. The separation of prostatic tissues into epithelium and stroma was performed according to the method of Cowan et al. [2] as modified by Krieg et al. [14] without using NaN_3 in the sample buffer. A schematic diagram of the separation is given in Fig. 1.

Other analytical procedures

SDS-polyacrylamide gel electrophoresis was carried out according to the method of Laemmli [15]. Protein concentrations were determined by the method of Bradford [1] using bovine serum albumin as a standard.

Quantitative determination of amino acids was carried out according to the method of Moore, Spackman and Stein [20, 24] with a Biotronic BT 6000 amino acid analyzer (Biotronik Wissenschaftliche Geräte, Frankfurt/M, FRG) on a cation exchange resin (Biotronik BTC 3118, Lot A 299). Fractions with growth factor activity after heparin-Sepharose chromatography demonstrating a single protein band in SDS-polyacrylamide gel electrophoresis were used for amino acid analysis.

Results

Purification of a growth factor from hyperplastic protatic tissues

The cytosol of BPH tissue (99 ml, protein concentration 2.0 mg/ml) was supplemented with 19 ml of a 3 mol/l NaCl solution to give a final NaCl concentration of 0.46 mol/l. The total growth factor activity of this solution was

Table 1. Purification of a growth factor from human hyperplastic prostatic tissues

	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)	Purification (-fold)
Crude extract	200.6	28320	141.2	100	1
Heparin-Sepharose chromatography	0.07	18750	267857	66	1897

Table 3. Growth factor activities in crude extracts of separated prostatic tissue

Fraction	Total protein (mg)	Total activity (units)	Specific activity (units/mg protein)	Relative activity referring to all fractions (%)
Stromal fraction (S)	30	2325	77.5	73.6
Epithelium fraction (E)	4.8	172	35.8	5.4
Epithelial supernatant (ES)	99.2	625	6.3	19.8
Stromal wash filtrate (FS)	94.5	38	0.4	1.2

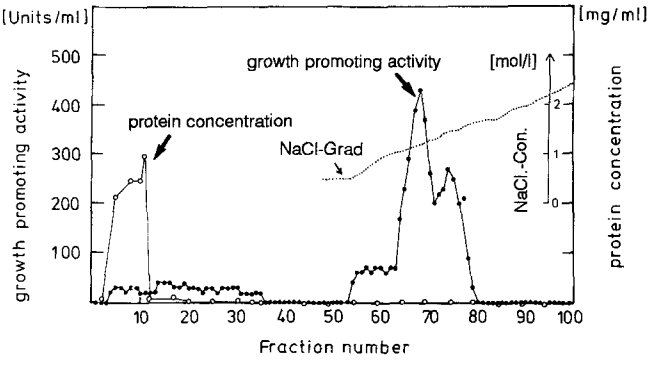

Fig. 2. Affinity chromatography of a growth factor from BPH tissues on a heparin-Sepharose column. Fraction volumes were 9 ml each from no. 1 to no. 13 and 5 ml each from no. 14 to no. 99. ●●●, Growth-stimulating activity; OOO, protein concentration; ———, NaCl concentration

28320 units (specific activity 142.2 units/mg protein). The solution was applied to a heparin-Sepharose column equilibrated with 10 mmol/l Tris-HCl (pH 7.5) containing 0.5 mol/l NaCl. It was found that 81% of growth factor activity was bound to the column; more than 99% of the protein remained in the unabsorbed fraction. The recovery of growth factor activity over all fractions was 95%. Growth factor activity was eluted with 1.10–1.55 mol/l NaCl. By this step, growth factor activity was purified about 1900-fold; the recovery was about 66%. A typical result is shown in Table 1 and Fig. 2. Further purification was achieved by combining active fractions and diluting the combined solution with 10 mmol/l Tris-HCl (pH 7.5) to give a final NaCl concentration of 0.8 mol/l, followed by heparin-Sepharose column chromatography in the presence of 10 mmol/l Tris-HCl (pH 7.5) containing 0.8 mol/l NaCl. After washing the column with the same buffer, the activity was eluted with 10 mmol/l Tris-HCl (pH 7.5) containing 2.0 mol/l NaCl. Growth factor activity was concentrated and further purified several-fold by this step.

Properties of the isolated growth factor

The molecular weight of the purified growth factor was estimated at 68000 by SDS-polyacrylamide gel electrophoresis. SDS gel electrophoresis demonstrated only a single protein band.

The amino acid composition determined is the result of several amino acid analyses. The amino acid composition of the purified growth factor is demonstrated in Table 2.

Distribution of growth factor activity in mechanically separated prostatic tissue

Prostatic tissue was separated into an epithelial and a stromal fraction as described above. The effectiveness of the separation procedure was controlled by light microscopic investigation of smear preparations of the epithelial and stromal fractions stained with a combined May-Grünwald-Giemsa staining. The epithelium fraction showed slightly damaged cells with round, darkly stained nuclei. There were only negligible amounts of stromal elements. The stromal fraction showed spindle-shaped nuclei in fibromuscular tissue with only negligible epithelial elements.

Krieg et al. [14] examined the degree of separation of epithelium and stroma by measuring hydroxyproline as a marker for collagenous connective tissue, i.e. stromal fraction, and acid phosphatase as a marker for epithelial cells. On examining 22 different BPH samples, they found that on average less than 6% of the epithelial acid phosphatase content was detectable in the stromal fraction. On the other hand, on average 17% of the stromal hydroxyproline content was determined in the epithelial fraction. Thus, the separation method provided material

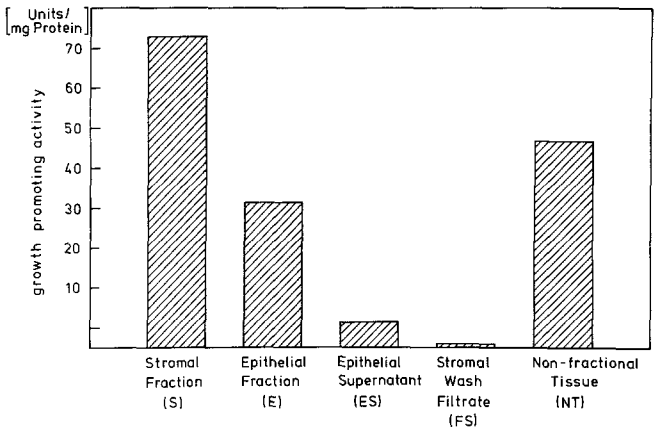

Fig. 3. Specific growth-stimulating activities in crude extracts of separated and unseparated prostatic tissue

Table 2. Amino acid composition of the isolated growth factor

Amino acid	Absolute amino acid number per molecule	Relative amino acid number per molecule (%)
Asx	36.6	6.7
Thr	27.8	5.1
Ser	52.9	9.7
Glx	57.3	10.5
Pro	17.5	3.2
Gly	73.7	13.5
Ala	49.1	9.0
Val	32.7	6.0
Cys	4.4	0.8
Met	7.1	1.3
Ile	24.6	4.5
Leu	43.1	7.9
Tyr	16.9	3.1
Phe	12.0	2.2
His	13.1	2.4
Trp	0–1	0–0.2
Lys	40.9	7.5
Arg	36.0	6.6
Total	548	100

of sufficient purity when examined by histological or biochemical methods.

Mechanically separated prostatic tissue was homogenized by a Potter-Elvehjem homogenizer at a setting of 4 for 1 min and 1-min intervals with cooling (3 times). Homogenized fractions were centrifuged at 38000 *g* for 1 h. The resulting supernatant was filtered through glass wool. Diluting the supernatants with 10 mmol/l Tris-HCl (pH 7.5) the protein concentration of all crude extracts was adjusted to 1.2 mg/ml. A crude extract of unseparated prostatic tissue was prepared in the same way. Growth factor activity of these fractions was measured as described above. The results of growth factor determination are shown in Fig. 3 and Table 3. The results demonstrate specific growth factor activity in the stromal fraction of mechanically separated prostatic tissue that was about two-fold that in the epithelial fraction. The specific growth-stimulating activities of the epithelial supernatant and the stromal wash filtrate were less than 8% and 0.5% respectively, of the stromal fraction. Referred to the total activity of whole tissue, about 74% of growth-stimulating activity was detected in the stromal fraction; only about 5% was detectable in the epithelial fraction.

Discussion

The results presented in this paper demonstrate the existence of growth factor activity in human hyperplastic prostatic tissues. The purification of a growth factor with a molecular weight of 68000 is reported. This growth factor may be identical or similar to one described by Lawson et al. [16] in crude extracts of hyperplastic prostatic tissues, whose molecular weight was estimated at about 67000 by gel filtration. The growth factor investigated showed a high affinity for heparin, a highly sulfated glycosaminoglycan. It may therefore be one of heparin-binding factors, a new class of polypeptide mitogens. As their only common biochemical property is high affinity for heparin, heparin affinity chromatography has become an essential step in their purification [17].

The purified growth factor presented in this paper was eluted with 1.10–1.55 mol/l NaCl. This concentration is comparable to those necessary for the elution of other known growth factors from several sources from heparin-Sepharose [5, 8, 18, 21, 23, 27, 28].

Since the first description of a growth factor in crude extracts of hyperplastic prostatic tissues [11], several investigators were able to demonstrate growth factors in human hyperplastic prostatic tissues [9, 12, 21, 26, 27, 29]. Different growth factors may probably exist in human prostatic tissues, because the physical and biochemical properties of known growth factors isolated from BPH are highly different. Their molecular weights vary from 13000 to 300000, and growth factors with either basic or acidic isoelectric points are described [11, 20, 25]. Precursor-product relations are thought to occur [29].

Comparison of the amino acid composition of the present growth factor with data of other growth factors of different origin [3, 7, 17] reveals no striking differences. A low content of the amino acids tryptophan and cysteine is notable. Overall, the unpolar amino acids predominate, and basic and acidic amino acids occur in nearly equal concentrations. No reason for the high affinity of the described growth factor to heparin is apparent from the amino acid composition. The amino acid composition and the molecular weight of the isolated growth factor were different from the data of other known growth factors. Preliminary sequencing of the peptide chain indicated a blocked N-terminus. Considering the available information, the growth factor presented here seems to be a new entity.

The growth factor investigated showed a striking distribution in prostatic tissue. Most of the growth factor activity was found in stromal tissue, and the minor part of the activity in the epithelial fraction.

It is also conceivable that the origin of the present growth factor is outside the prostate gland, e.g. in the hypothalamus or the pituitary gland. The growth factor may be transported via the bloodstream to the site of action. The effect of the growth factors on the differentiation and growth regulation of the prostate gland may be exerted with the help of growth factor receptors, which have not yet been demonstrated. A different distribution of growth factor receptors in the prostate gland could explain the striking distribution of growth factor activity in prostatic tissue.

Up to now, the role of growth factors in the pathogenesis of BPH is unclear. However, in early 1939, Deming and Neumann [6] postulated that the stromal tissue of the prostate might produce some stimulating and proliferating effects on the epithelium. Such an effect may be exerted by growth factors. Cunha [4] supposed that the response of epithelial cells on androgenes might be produced from stroma cells by trophic influences, which might serve as target cells for androgens. Furthermore, McNeil [19] postulated that a trophic inducing factor from stroma might cause epithelial cell proliferation. Growth factors could act as proliferating and inducing agents of this kind. Stroma cells may be caused to produce large amounts of growth factors by some unknown influence. These growth factors may have an important influence on normal cell growth and replication. Large amounts of this growth factor may cause a hyperplasia of stromal tissue by autocrine and paracrine mechanisms with subsequent epithelial cell proliferation. This hypothesis takes account of the well-known fact that BPH begins with proliferation of fibroblasts and that epithelial elements are only secondarily affected. The possible origin of growth factor in stromal tissue is supported by the fact that the major part of growth factor activity is localized in stromal tissue of BPH. The role of growth factors in the pathogenesis of BPH is only hypothetical. Other etiologic factors, e.g. steroid hormones and stroma-epithelium interactions may also play a role. A differential expression of growth factor activity in fetal, adolescent and normal adult prostate as well as in BPH could help to explain the participation of growth factors in the pathogenesis of BPH. Until now, there is conflicting information about the expression of growth factors in normal prostatic and BPH tissues. Jinno et al. [12] and Hierowski et al. [9] were able to demonstrate quantitative differences of growth factor activity between normal and BPH tissues. In contrast, Jacobs et al. [11], Lawson et al. [16] and Tackett et al. [29] were not able to demonstrate quantitative differences. Nevertheless, qualitative differences in growth factors that may at present be beyond the reach of detection because of a lack of sensitive methods to determine growth factor activity, may play a role in pathogenesis of BPH.

Nevertheless, further investigation is necessary before the role of growth factors in the pathogenesis of BPH will be understood.

Acknowledgement. The authors wish to thank Mrs. C. Doll for skillfull technical assistance and Prof. Dr. M. Krieg (Institute of Clinical Chemistry, University Clinic "Bergmannsheil", Bochum) for valuable discussion. Furthermore the authors are indebted to Prof. Dr. T. Senge (Dept. of Urology, "Marienhospital", Herne, University of Bochum) and Prof. Dr. K. M. Müller (Dept. of Pathology, University Clinic "Bergmannsheil", Bochum) for supplying us with prostates.

References

1. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248
2. Cowan RA, Cowan SK, Giles CA, Grant JA (1976) Prostatic distribution of sex hormone-binding globulin and cortisol-binding globulin in benign hyperplasia. J Endocrinol 71:121
3. Crabb JW, Armes LG, Carr SA, Johnson CM, Roberts GD, Bordoli RS, McKeehan WL (1986) Complete primary structure of prostatropin, a prostate epithelial cell growth factor. Biochemistry 25:4988
4. Cunha GR, Chung LWK, Shannon JM, Reese BA (1980) Stromal-epithelial interactions in sex differentiation. Biol Reprod 22:19
5. D'Amore PA, Klagsbrun M (1984) Endothelial cell mitogens derived from retina and hypothalamus: biochemical and biological similarities. J Cell Biol 99:1545
6. Deming CL, Neumann C (1939) Early phases of prostatic hyperplasia. Surg Gynecol Obstet 68:155
7. Esch F, Baird A, Ling N, Ueno N, Hill F, Denoroy L, Klepper R, Gospodarowicz D, Bohlen P, Guillemin R (1985) Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino terminal sequence of bovine brain acidic FGF. Proc Natl Acad Sci USA 82:6507
8. Gospodarowicz D, Cheng J, Lui G-M, Fujii DK, Baird A, Bohlen P (1985) Fibroblast Growth Factor in human placenta. Biochem Biophys Res Commun 128:554
9. Hierowski MT, McDonald MW, Dunn L, Sullivan JW (1987) The partial dependency of human Growth Factor on steroid hormones in stimulating thymidine incorporation into DNA. J Urol 138:909
10. Huggins C, Stevens RA (1940) The effect of castration on benign hypertrophy of the prostate in man. J Urol 43:705
11. Jacobs SC, Pikna D, Lawson RK (1979) Prostatic Osteoblastic Factor. Invest Urol 17:195
12. Jinno H, Ueda K, Otaguro K, Kato T, Ito J, Tanaka R (1986) Prostate Growth Factor in the extracts of benign prostatic hypertrophy. Eur Urol 12:41
13. Klagsbrun M, Langer R, Levenson R, Smith G, Lillehei C (1977) The stimulation of DNA synthesis and cell division in chondrocytes and 3T3 cells by a growth factor isolated from cartilage. Exp Cell Res 105:99
14. Krieg M, Klötzl G, Kaufmann J, Voigt KD (1981) Stroma of human benign prostatic hyperplasia: preferential tissue for androgen metabolism and estrogen binding. Acta Endocrinol (Copenh) 96:422
15. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680
16. Lawson RK, Story MT, Jacobs SC (1981) A growth factor in extracts of human prostatic tissue. In: Murphy GP, Sandberg AA, Karr JP (eds) The prostatic cell: structure and function. Liss, New York, p 325
17. Lobb RR, Harper JK, Fett JW (1986) Purification of Heparin-Binding Growth Factors. Anal Biochem 154:1
18. Matuo Y, Nishi N, Matsui SJ, Sandberg AA, Isaacs JT, Wada F (1987) Heparin binding affinity of Rat Prostatic Growth Factor in normal and cancerous prostates: partial purification and characterization of Rat Prostatic Growth Factor in the Dunning tumor. Cancer Res 47:188
19. McNeil JE (1978) Origin and evolution of benign prostatic enlargement. Invest Urol 15:340

20. Moore S, Spackman DH, Stein WH (1958) Chromatography of amino acids on sulfonated polystyrene resins: an improved system. Anal Chem 30:1185
21. Nishi N, Matuo Y, Muguruma Y, Yoshitake Y, Nishikawa K, Wada F (1985) A human prostatic growth factor (hPGF): partial purification and characterization. Biochem Biophys Res Commun 132:1103
22. Nishikawa K (1982) Intracellular DNA synthesis factor from rat rhodamine fibrosarcoma. Biochem Int 4:169
23. Shing Y, Folkman J, Sullivan R, Butterfield C, Murray J, Klagsbrun M (1984) Heparin affinity: purification of a tumor-derived capillary growth factor. Science 223:1296
24. Spackman DH, Stein WH, Moore S (1958) Automatic recording apparatus for use in the chromatography of amino acids. Anal Chem 30:1190
25. Story MT, Jacobs SC, Lawson RK (1984) Preliminary characterization and evaluation of techniques for the isolation of prostate-derived growth factor. Prog Clin Biol 145:197
26. Story MT, Jacobs SC, Lawson RK (1984) Partial purification of a prostatic growth factor. J Urol 132:1212
27. Story MT, Sasse J, Jacobs SC, Lawson RK (1987) Prostatic Growth Factor: purification and structural relationship to basic Fibroblast Growth Factor. Biochemistry 26:3843
28. Sullivan R, Klagsbrun M (1985) Purification of cartilage-derived growth factor by heparin-affinity chromatography. J Biol Chem 260:2399
29. Tackett RE, Heston WDW, Parrish RF, Pletscher LS, Fair WR (1985) Mitogenic factors in prostatic tissue and expressed prostatic secretion. J Urol 133:45
30. Walsh PC (1976) Benign prostatic hyperplasia: etiological considerations. Prog Clin Biol Res 6:1
31. Walsh PC (1984) Human benign prostatic hyperplasia: etiological considerations. Prog Clin Biol Res 145:1
32. Wilson JD (1980) The pathogenesis of benign prostatic hyperplasia. Am J Med 68:745
33. Wilson JD (1987) The testes and the prostate – a continuing relationship. N Engl J Med 317:628

Dr. A. Dignaß
Institut für Physiologische Chemie
Abteilung für Biochemie des Intermediärstoffwechsels
Ruhr-Universität
W-4630 Bochum 1
Federal Republic of Germany