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Polyamines, Hydrolases (PAP, LAP, SDH, Plasmin) TSH, T_3, T_4 and C-Peptide in Benign Hyperplasia of the Prostate*

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Summary. High concentrations of polyamines have been found in the normal human prostate. The profile of these amines appeared significantly changed in benign hypertrophy of the prostate. An increase of spermine and a fall of putrescine were always found in patients with a hypertrophied prostate weighing more than 30 g. Alterations of plasmin in these tissues seemed to reflect changes in the matrix; abnormalities of thyroid and pancreatic function documented by changes in the serum levels of TSH and c-peptide which are thought to be further evidence of a mesenchymal-epithelial interaction in the pathogenesis of benign prostate hypertrophy.

Key words: BPH, Polyamines, Hydrolases, (PAP, LAP, SDH, plasmin), TSH, T_3, T_4, C-peptide.

INTRODUCTION

The aetiology of benign prostatic hypertrophy (BPH) and its evolution remain unsettled despite the abundance of previous research (1, 2, 3).

Endocrine inbalances, such as changes in androgens, oestrogens (4, 5) and testosterone binding globulin (6), with ageing do not occur at the same time as the appearance of nodules in the transitional zone of the prostate (7).

In most men under the age of 70 nodules develop, but the main cause of prostatic enlargement seems to be a diffuse growth of the transition zone. Further development is dominated by massive enlargement and glandular proliferation (7). All studies appear to accept a stimulated growth of these prostatic nodules due to either a hormonal influence or by some humoral inducing agents from the stroma (8); degradation and phagocytosis are completely disregarded.

It is the purpose of this investigation to analyse growth rate and enzymatic activity of cells in normal and benign hyperplasia of the prostate by two different approaches, the polyamine assay and histochemistry of tissue samples.

Polyamines are low molecular weight compounds that specifically regulate DNA replication (9) and the content of ribosomal RNA (10) and are found in abundance in the prostate (11).

Prostatic acid phosphatase, leucine aminopeptidase, mitochondrial succinate dehydrogenase and plasmin were measured for evidence of associated pathological findings in BHP.

It has been observed that maturity onset diabetes occurs more frequently in patients with BHP than in age-matched control groups (12, 13, 14). Hypothyroidism, another endocrine pathology, has a high incidence in elderly patients (15). Thus, it was also of interest to study by radioimmunassay c-peptide, (an equimolar split product of proinsulin) TSH, T_3 and T_4.

MATERIAL AND METHODS

Normal prostatic tissue was obtained from kidney donors (n = 2) and from young males (n = 3) after accidental death. Tissue was stored at -30°C. Prostatic tissue exhibiting benign hypertrophy (n = 23), established histologically, was obtained from open prostatectomy and frozen after operation at -30°C. The gland weight ranged from 35-150 g.

Tissue Preparation

Tissues were homogenised in 4 volumes of cold 10% TCA and centrifuged at 50,000 x g for 20 min.

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The supernatant was diluted 1:20 so that polyamine concentrations were close to 200 pmoles/20 µl. Standards of 200 pmoles/20 µl of each amine were run routinely, and trace amounts of (14C) polyamines were added to assess recovery rates. Recovery rates were consistently 100%.

Analysis Procedure for Polyamines

Analyser: A Durrum D-500 amino acid analyser (Durrum Instrument Corp., Sunnyvale, CA) equipped with a fluorescence detector assembly with a 2-mm path-length flow cell was used for polyamine analysis. All sample functions, including injection and peak area analysis, were done by a PDP 8/M computer. As many as 80 sample cartridges could be loaded for automatic analysis and injection. Resin, buffers, fluorescent reagent and elution programme have been described elsewhere (16, 17).

Histological Techniques

Immediately after surgery the tissue samples of benign hyperplasia of the prostate and of anaplastic carcinoma were frozen in carbon dioxide at -76°C and stored. Cryostate sections of 10 µm were prepared and incubated in mixtures for PAP, LAP and SDH (18, 19, 20). Plasmin was stained after incubation in benzoylarginine-nitranilide and polymerisation of metacrylate (21).

Radioimmunassay Procedures

Venous blood samples were collected before ingestion of food. Blood was defibrinised by glass beads, spun down at 500 rpm and the supernatant was stored at -30°C until assayed by a double antibody technique (BykMallinckrodt, Dietzenbach, West-Germany). In β -cells of the pancreas the splitting of pro-insulin by proteolytic enzymes yields c-peptide and insulin. Though the biological function of c-peptide is unknown, it is possible to measure the secretory rate of insulin by determination of the serum level of c-peptide. Yanaihara succeeded in synthesising a peptide which was identical to human c-peptide (22). Antibody cross reacted immunologically neither with porcine nor bovine c-peptide nor with human insulin.

The intra-assay variation coefficient for TSH was 5.5%, for T_3 was 6%, for T_4 was 4.3% and for c-peptide was 5%. The inter-assay variation coefficient for TSH was 14%, for T_3 was 14.6%. for T_4 was 5.2% and for c-peptide was 12%.

Control Group and Statistical Analysis

Age matched control groups for patients with BPH are difficult to establish. Tissue specimens of the prostatic capsule of elderly patients are not satisfactory normal tissue controls. Thus, we decided to take normal prostate from kidney donors, although these patients were not comparable in respect of age and hormonal situation.

Serum levels of c-peptide, TSH, T_3 and T_4 in patients with BPH were compared to patients of the same age but without clinical signs of BPH (spincter-colliculus distance less than 2.0 cm).

The results were analysed by paired and unpaired t-tests, coefficient of correlation and spearman r.

RESULTS

Polyamine Concentrations in Normal and Hyperplastic Prostate

Normal human prostate had high concentrations of putrescine and spermine with intermediate spermidine concentrations (Table 1). In patients with BPH there was a significant increase of spermine ($p = 0.01$).

Although not highly significant, spermidine concentrations were slightly elevated whereas the content of putrescine was decreased ($p = 0.05$) (Table 1).

The growth rate of tissues can be calculated by means of plotting putrescine levels versus spermidine/spermine levels. In 62% of all cases investigated the growth rate of hyperplastic prostates appeared clearly reduced in comparison to normal prostate from kidney donors (Fig. 1). No relation to age was proved.

Histochemistry of PAP, LAP, SDH and Plasmin in Normal and Hyperplastic Prostate

PAP is present in large amounts in the glandular component of both normal and hyperplastic

Table 1. Polyamines in hyperplastic tissue specimen of prostate are shown to have significant differences in the content of spermine ($p = 0.01$). The level of putrescine is distinctly reduced by hyperplasia of the prostate, whereas spermidine is biphasically modulated

Polyamines in Prostate

	Normal nmoles/ mg protein	BPH nmoles/ mg protein	
Putrescine	10 ± 3.5	7.1	95% ↓
Spermidine	4.3 ± 0.8	5.3	43% ↑
		2.7	30% ↓
Spermine	27 ± 5.1	33.0	87% ↑

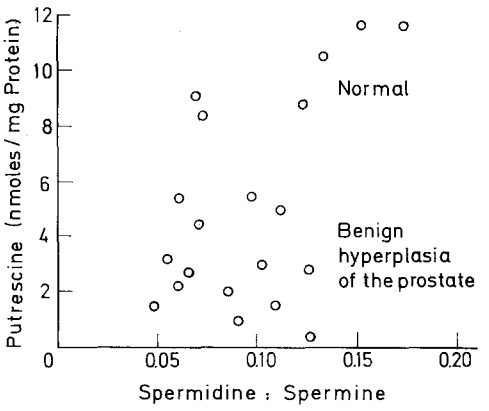

Fig. 1. By plotting putrescine versus spermidine/spermine information about the growth rate can be obtained. This rate appears to be clearly lowered in patients with BPH

prostates (Figs. 2, 3). No change in activity appeared in epithelial cells of BPH when compared to normal prostatic cells. Leucine-aminopeptidase was not significantly changed in hyperplastic or hypertrophied cells and showed no difference to normal prostatic cells (Figs. 4, 5). By means of a histochemical technique SDH was detected in similar concentrations in epithelial cells of normal and hyperplastic prostates and to a lesser extent in the connective tissue of BPH (Figs. 6, 7).

After incubation with a synthetic dipeptide, plasmin activity could be measured. The enzymatic activity was mainly demonstrated in the matrix component of the prostate and appeared to be distinctly lower in BPH (Figs. 8, 9).

Serum Level of C-Peptide, TSH, T_3 and T_4 in Patients with BPH and in an Age Matched Control Group

Serum from 43 patients suffering from BPH was analysed for c-peptide, TSH, T_3 and T_4. The results were compared to the serum level in 46 patients of similar age. Elevated concentrations of c-peptide were found in 20 patients (49%) whereas in the age matched control group (n = 46) an elevated serum level was found in 20%. Morning blood sugar values of all patients investigated did not exceed 140 mg% and no patients received antidiabetic drugs (Fig. 10).

The concentration of serum TSH in patients with BPH was above normal (3 µIU/ml) in 45% of all tested sera; the control groups were in the normal range (Fig. 10). Serum levels of T_3 and T_4 were not significantly changed and no correlation between TSH and T_3 or T_4 could be established (Fig. 11).

DISCUSSION

It is evident that there is a multifactorial pathogenesis of BPH and that pathological imprints change in the course of the disease.

Our data suggest that polyamines accumulate above normal levels in BPH. The fact that polyamines are found in large amounts in cells, contrasting with only traces in blood and other body fluids, is sufficient evidence to attribute the increase of spermine in BPH to the glandular component of the prostate.

In the normal prostate there is a slow rate of DNA synthesis (23) but a higher rate in BPH. The enzymatic pattern of hyperplastic and hypertrophied prostatic glandular cells did not seem to be affected by this.

Generally, homeostatic control initiating DNA synthesis and cell proliferation and degradation prevents normal tissue from hyperplasia and neoplasia.

In BPH a dissociation of this mechanism may be present, suggested by the finding of stimulated DNA synthesis - as indicated by the increase of spermine - of reduced growth rate - as suggested by the lowered ratio of spermidine/spermine in comparison to intact prostate - and by altered tissue fibrinolysis, one factor of degradation - as measured by the histochemistry of plasmin.

Common diseases in elderly patients, such as maturity-onset diabetes and hypothyroidism may be only incidentally related to BPH. To maintain prostatic cells in culture, however, adequate concentrations of insulin are necessary (24). A further functionally important role of insulin, T_3 and T_4 (or indirectly of TSH) is to sustain metabolism of connective tissues (25). In this study serum of patients with BPH has been shown to have an aberrant content of c-peptide and TSH suggesting a pre-diabetic state and compensated hypothyroidism. Changes in stromal metabolism are thought to precede BPH (8, 26) and this may be partially caused or aggravated by altered pancreatic and thyroid function.

Though it is not possible to define exactly the stage of BPH the results presented here allow the following conclusion. Established BPH is reflected by a high content of spermine, a low ratio of spermidine/spermine and a decrease in plasmin, whereas in earlier stages of the disease changes in these factors are not so distinct.

Alterations in the rate of secretion of insulin, TSH and testosterone are thought to have an influence on benign enlargement of the prostate.

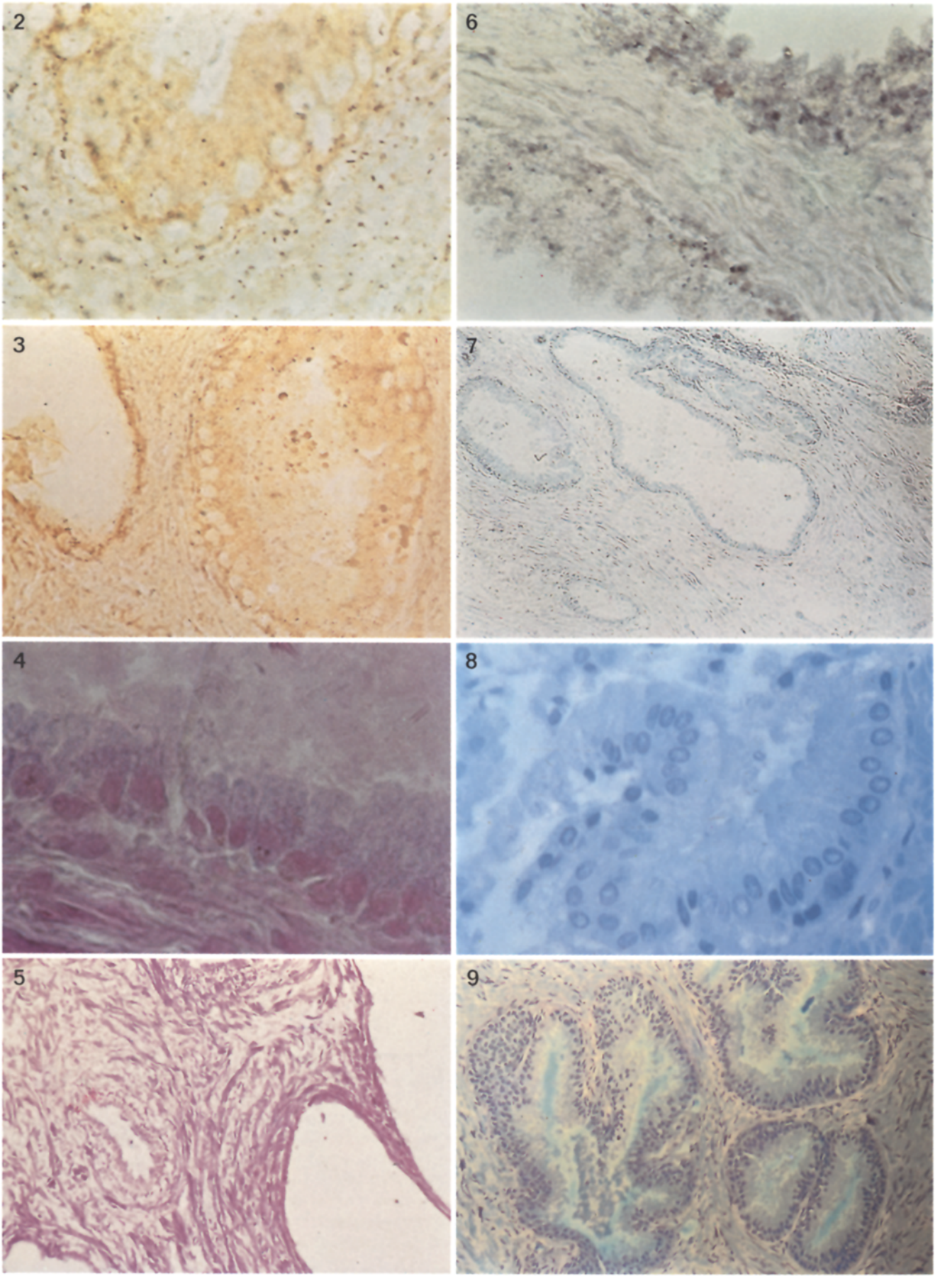
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Fig. 2. Prostatic acid phosphatase in normal tissue samples of the prostate is mainly present in the apical district of the epithelial cells. (Substrate: Na-napthylphosphate) (1150 x)

Fig. 3. PAP in the glandular component of BPH in stained in similar pattern and it is supposed to have the same activity of PAP in comparison to normal prostatic tissues (860 x)

Figs. 4, 5. Leucine aminopeptidase, a zinc dependent enzyme, can be easily shown by incubation of c-leucine β-napthylamid in both normal and hyperplastic tissues (1150 x, 860 x)

Figs. 6, 7. SDH is found in mitochondrial matrix and associated to the citrate cycle. It can be demonstrated by histochemical means especially in the epithelial cells and to some extent in the stromal component of normal and hyperplastic prostate without difference (Substrate: succinate (cystalline)-dinatrium) (1250 x, 420 x)

Figs. 8, 9. Plasmin activity is evaluated by inhibition of a synthetic dipeptide (BAPA) and increasing concentrations of plasmin inhibitors (21). The content of plasmin in BHP is reduced and can be inhibited by antifibrinolytic agents which contrasts with the negative inhibition in normal tissues. Inhibited tissue specimen are detected by metachromasy (Fig. 9) (420 x, 420 x)

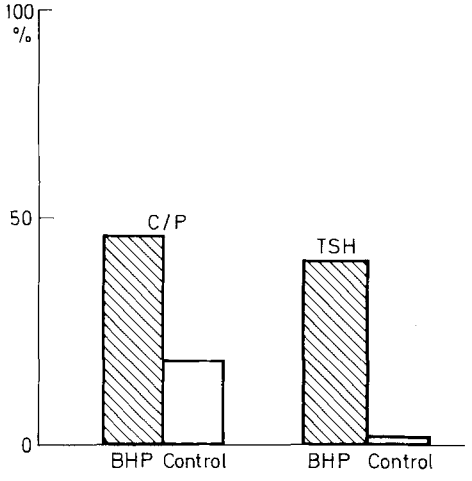

Fig. 10. Serum level of c-peptide (an equimolar split product of proinsulin) and TSH are depicted in patients with BPH and an age matched control group. Elevated serum level of c-peptide suggest a prediabetic situation and appear to be more frequent in BPH. Serum level of TSH are significantly higher in patients with BPH than in a control group ($p = 0.01$)

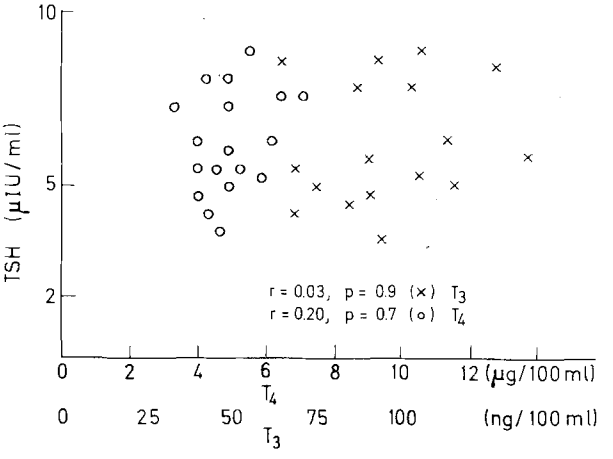

Fig. 11. Increased serum levels of TSH do not correlate with serum levels of T_3 and T_4, suggesting compensated hypothyroidism in this group

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