Gastricsin in the benign and malignant prostate

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SUMMARY An immunoperoxidase (peroxidase-antiperoxidase) method was used to localise the gastric acid proteinase gastricsin in prostate. The enzyme was present, probably as zymogen, in acinar lining cells in 66 (69%) of 96 cases of benign prostatic enlargement; other normal tissues from male genital tract were negative. It was also present in the tumour cells in 21 (39%) of 54 cases of prostatic adenocarcinoma. The findings support the suggestion that the prostate is the source of the gastricsin of normal seminal fluid. It is not yet clear whether its presence in prostatic carcinomas will be of diagnostic use.

Human gastric juice contains a number of acid proteinases, the main ones in adults being pepsin and gastricsin. Both of these enzymes have molecular weights of about 35 000, but gastricsin has a higher pH optimum.⁴ They are also immunologically distinct.⁵ The enzymes are produced in the form of zymogens (pepsinogen and progastricsin, respectively), which undergo conversion into their active forms on exposure to the low pH in the gastric juice.⁶ Pepsinogen is the main zymogen found in the gastric body; progastricsin is produced primarily in the gastric body and antrum but also in the duodenum.⁶

Seminal fluid also contains an acid proteinase.⁷ This has been purified from human seminal plasma and an enzyme with analogous activity has been isolated from homogenates of prostate.⁸ Recently, we have characterised this enzyme biochemically and identified it as a gastricsin by showing immunological identity with gastric gastricsin and total lack of cross reactivity with an antiserum to human pepsin.⁷ Using the immunoperoxidase (peroxidase-antiperoxidase) technique on formalin fixed, paraffin embedded surgical resection specimens we have established the origin of the enzyme in the acinar epithelium of the prostate. In order to consider the possibility that this enzyme might be produced in the neoplastic prostate, the study was extended to include a series of prostatic carcinomas.

Material and methods

PREPARATION OF ANTISERA
Pepsin and gastricsin purified from human gastric juice/mucosa were generous gifts from Dr AP Ryle and Professor J Tang respectively. Progastricsin was purified from human seminal fluid, as described previously.⁷ Pig pepsin was obtained from Sigma Chemicals. Rabbit antiserum to pepsin and gastric gastricsin were prepared as described previously.⁷

Rabbit antiserum to human prostatic acid phosphatase was purchased from Miles Laboratories Ltd.

USE OF ANTISERA
Antigastricsin was used at a dilution of 1/100 in 10% normal human serum in 0-05 M Tris buffer, pH 7-6, for 30 min; antipepsin was used at a dilution of 1/200 in the buffer only. Paraffin embedded sections (6 μm) were stained with the immunoperoxidase (peroxidase-antiperoxidase) method⁹ and counterstained with haematoxylin. For absorption experiments, seminal zymogen or enzyme and both preparations of gastric pepsin and gastricsin were incubated with the antiserum for 1 h at room temperature before use (final concentration 10 μg/ml dilute antiserum).

Antiprostatic acid phosphatase was used at a dilution of 1/500 under similar conditions to those for antipepsin.

TISSUES
All tissues were from the routine surgical files, Glasgow Royal Infirmary, and had been fixed in 10% unbuffered formalin and embedded in paraffin.
Experiments with a variety of fixatives show that this gives the best preservation of gastricsin, with the exception of Bouin's fixative. Ninety six cases of benign enlargement of the prostate were studied: in 85 of these the prostate had been removed by transurethral resection, and in the others suprapubic enucleation of the prostate was performed. A block of normal stomach was used as a positive control. Other normal tissues studied were testis (16), epididymis (12), vas deferens (11), seminal vesicle (1), and bladder transitional mucosa (10).

In addition, 54 cases of prostatic carcinoma were studied. In 44 cases the prostate had been removed by transurethral resection, and in the other 10 by suprapubic enucleation.

The specificity of staining by each antiserum has been described previously. Absorption controls of antigastricsin incubated with gastricsin and of antigastricsin incubated with pepsin were performed on a selected group of the most strongly positive 13 cases of benign nodular hyperplasia and 12 cases of carcinoma of the prostate. Pig pepsin was used for the pepsin absorption studies since it is immunologically similar to the human enzyme and human pepsin was in short supply.

Each batch of slides stained included a positive control section of normal gastric mucosa and, where appropriate, an absorption control section, also of stomach.

A selected group of nine carcinomas, four including areas of benign prostate, was further studied by staining alternate serial sections for gastricsin and prostatic acid phosphatase. These were examined with a comparison microscope and the distribution of the two enzymes was compared. Ten selected cases of benign nodular hyperplasia were similarly examined. A selected group of 19 benign prostates was stained with antipepsin.

**Results**

**SPECIFICITY OF ANTISERA FOR GASTRICSIN OR PEPsin**

Normal gastric mucosa showed strong staining for gastricsin in chief cells and mucous neck cells and in the antral glands, as well as in some of the Brunner's glands in the duodenum. The pattern of staining was similar to that described by Samloff and Liebman. The specificity of staining has previously been discussed in detail. Staining of gastric mucosa was completely abolished by prior incubation of antigastricsin with gastric gastricsin, seminal gastricsin, or

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Fig. 1. Benign prostate stained for gastricsin (peroxidase-antiperoxidase method). Note gastricsin in acinar cells. × 76.
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semenal progastricsin. In contrast, pig pepsin, analogous to human pepsin, failed to block staining when used at the same concentration (10 μg/ml) or even at a much higher concentration (1 mg/ml). With antipepsin the gastric chief cells and mucous neck cells stained strongly. This could be abolished by absorption with pig pepsin but not with seminal or gastric gastricsin or seminal progastricsin.

BENIGN PROSTATES
Sixty six of 96 cases of benign enlargement of prostate showed staining for the gastricsin in the acinar lining cells. Staining was often confined to a few acini only but was always strong (Fig. 1). In other cases staining was more widespread, but invariably it was focal. Often supranuclear staining of secretion was apparent in the tips of cells (Fig. 2). Prostates removed intact showed no difference in staining from those removed by transurethral resection.

All sections of normal testis, epididymis, vas deferens, seminal vesicle, and bladder transitional mucosa were negative.

Control sections of normal gastric body included with each batch of slides were always positive and the corresponding absorption controls were negative.

MALIGNANT PROSTATES
Of the 54 cases of carcinoma studied, 21 showed gastricsin in the cytoplasm of the neoplastic cells. The staining was always intense but the pattern was focal. In some cases only a few cells were positive whereas in many others, staining was more extensive (Fig. 3). All positive tumours included at least some areas showing the typical microacinar pattern seen in prostatic carcinoma. In nine cases, however, there were undifferentiated areas; in four of these cases the staining showed a tendency to be in the less well differentiated areas. No such preponderance was seen in the other five cases.

SPECIFICITY OF STAINING OF PROSTATES
In the present series some of the more strongly staining benign and malignant cases were selected for absorption studies; 13 benign prostates and 12 carcinomas were examined. In every case staining was completely abolished by prior incubation of antigastricsin with gastric gastricsin (10 μg/ml). Similar preincubation of antigastricsin with pig pepsin at the same concentration, however, produced no significant reduction in staining. Sections of 19 of the most strongly gastricsin positive benign prostates were stained with antipepsin with negative results.

PROSTATIC ACID PHOSPHATASE STAINING
The nine prostatic carcinoma cases which had shown the most extensive staining for gastricsin were also examined for acid phosphatase distribution. All nine showed extensive strong staining for acid phosphatase in the cytoplasm of the malignant cells. Comparison microscopy of sections stained alternately for gastricsin and acid phosphatase showed that in
many instances most of the cells which contained gastricsin also stained for acid phosphatase. In general, however, acid phosphatase was distributed more widely, since many of the cells which contained this enzyme were negative for gastricsin. Ten cases of gastricsin positive benign prostates were similarly examined. In all cases nearly all acinar cells were acid phosphatase positive. Generally, staining was strong, although some cells were weakly stained and a few were negative. Comparison microscopy showed that in the benign glands all gastricsin positive cells contained acid phosphatase but not vice versa.

Discussion

Gastricsin was present in the acinar epithelium of 69% of 96 cases of benignly enlarged prostates. This would appear to be the source of the enzyme in seminal fluid since other possible sites of origin in the male genital tract were negative. Staining was always focal, with only some acini being positive, and within each acinus often only some cells were stained. The number of positive cells varied. Fresh normal human prostate is difficult to obtain, but it is likely that the observations made on the hyperplastic gland apply also to the normal gland and that gastricsin is a secretory product of the normal prostate.

The results of absorption studies are of interest. In all cases tested staining could be blocked by prior incubation of antiserum with antigen. Similar degrees of absorption were obtained with gastricsin prepared from seminal fluid and from stomach. The fact that absorption also occurred with the seminal fluid zymogen progastricsin indicates that there is a degree of immunological similarity between the zymogen and the active enzyme. It is difficult to state whether this immunological identity is complete or only partial because only small amounts of progastricsin were available and for this reason an experiment to compare the effectiveness of different titres of the two antigens in blocking antiserum could not be performed. Presumably, however, it is the proenzyme which stains in the prostatic acinar epithelium.

By contrast, the prostatic enzyme does not appear to be pepsin since: (a) no prostates were stained by the antiserum to human pepsin, and (b) staining of prostates by antigastricsin could be blocked with gastricsin but not with pepsin at the same or even much higher concentration. Cross absorption experiments between either antipepsin and gastricsin or antigastricsin and pepsin showed no recognisable reduction in staining.

Production of gastricsin by the benign prostate is reproduced in the malignant gland in 39% of cases studied. As in the benign gland, staining was focal in the carcinoma but was generally more extensive. No particular histological pattern appeared to be specifically associated with the presence of gastricsin, although in the positive cases the staining
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tended to be in the more poorly differentiated areas.

In a previous study we stained tissue from 24 cases of prostatic carcinoma with antipepsin; the results were negative.8

Acid phosphatase staining in prostatic carcinoma and benign enlarged prostates was similar to that obtained by other workers,12 although a recent study comparing staining of prostatic specific acid phosphatase and prostate specific antigen obtained a lower positivity rate.13 The serum level of acid phosphatase is raised in prostatic carcinoma, and it is likely that there is a similar increase of the serum or plasma gastricsin value. Whether or not its estimation will be of practical use in the diagnosis or management of prostatic carcinoma is not known; we are carrying out experiments to explore this possibility.

References


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