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Altered Distribution of Acid Phosphatase in Neoplastic Prostatic Cells

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Summary. Because of the well-known problem of variable cell differentiation encountered in the electron-microscopic evaluation of prostatic cancer, histochemical ultrastructural studies have been performed to assess whether an altered intracellular distribution of acid phosphatase is a more reliable index of malignant change. The results indicate that acid phosphatase activity not restricted to lysosomes is common in malignant cells, and that it may be an intermediate stage in the release of the enzyme into the serum.

Key words: Ultrastructure of prostatic cancer cells; Histochemical assessment of acid phosphatase; Release of acid phosphatase from lysosomes.

INTRODUCTION

Morphological diagnosis in prostatic cancer is difficult because of the high degree of cellular differentiation encountered in many cases (6, 11, 21). Diagnostic criteria have often been based on tissue architectural rather than intra-cellular alterations (11).

In an attempt to develop more reliable morphological methods, a histochemical study of acid phosphatase at the ultrastructural level has been performed with a view to obtaining an indication as to the functional integrity of lysosomes in prostatic cancer cells. Using the clinical observation of an increased serum activity of tartrate-labile acid phosphatase in cases of advanced prostatic cancer, we have attempted thereby to follow the pathway taken by acid phosphatase from its normal location in the lysosomes to the extracellular space.

MATERIAL AND METHODS

In 12 cases tissue was removed with a tru-cut needle (Travenol) biopsy, or in 2 cases by open operation. 4 biopsies were from cancerous tissue and 8 were taken from benign prostatic hyperplasia (BPH). The serum activities of tartrate-inhibitable acid phosphatase were 70, 30, 25, and 5 IU in the cases of cancer and always below 1.5 IU in BPH-cases determined by means of standard method (24).

A part of each tissue removed was used to confirm the diagnosis by light microscopy. Each biopsy was investigated with both staining methods (see below). Ten blocks were made from each tissue specimen. Samples were sliced from each tissue block and layered in equal amounts on 3 grids from every block.

The samples were immediately placed in 3% glutaraldehyde buffer in 0.1 M cacodylate, pH 7.4 at 0-4°C for 2 hours, for purposes of fixation and to prevent redistribution of acid phosphatase molecules (12).

The best fixation was obtained when the tissue was prepared in slices which have a square cross-section of sides 1 mm and were 1 cm long. The tissue was next removed from the glutaraldehyde solution and rinsed in 0.1 M phosphate buffer (pH 7.2). Cubes of sides 1 mm were cut. These were rinsed again in the phosphate buffer. The tissue was placed in the standard Palade fixative which was made up fresh as a 1% osmium tetroxide solution in 2% barbitone buffer. Finally the blocks were rinsed in 0.05 M Na-acetate buffer (16). Dehydration was performed with ethanol in the range between 50-100% (12). For block embedding we used Spurr's Resin (ERL 4206) (12).

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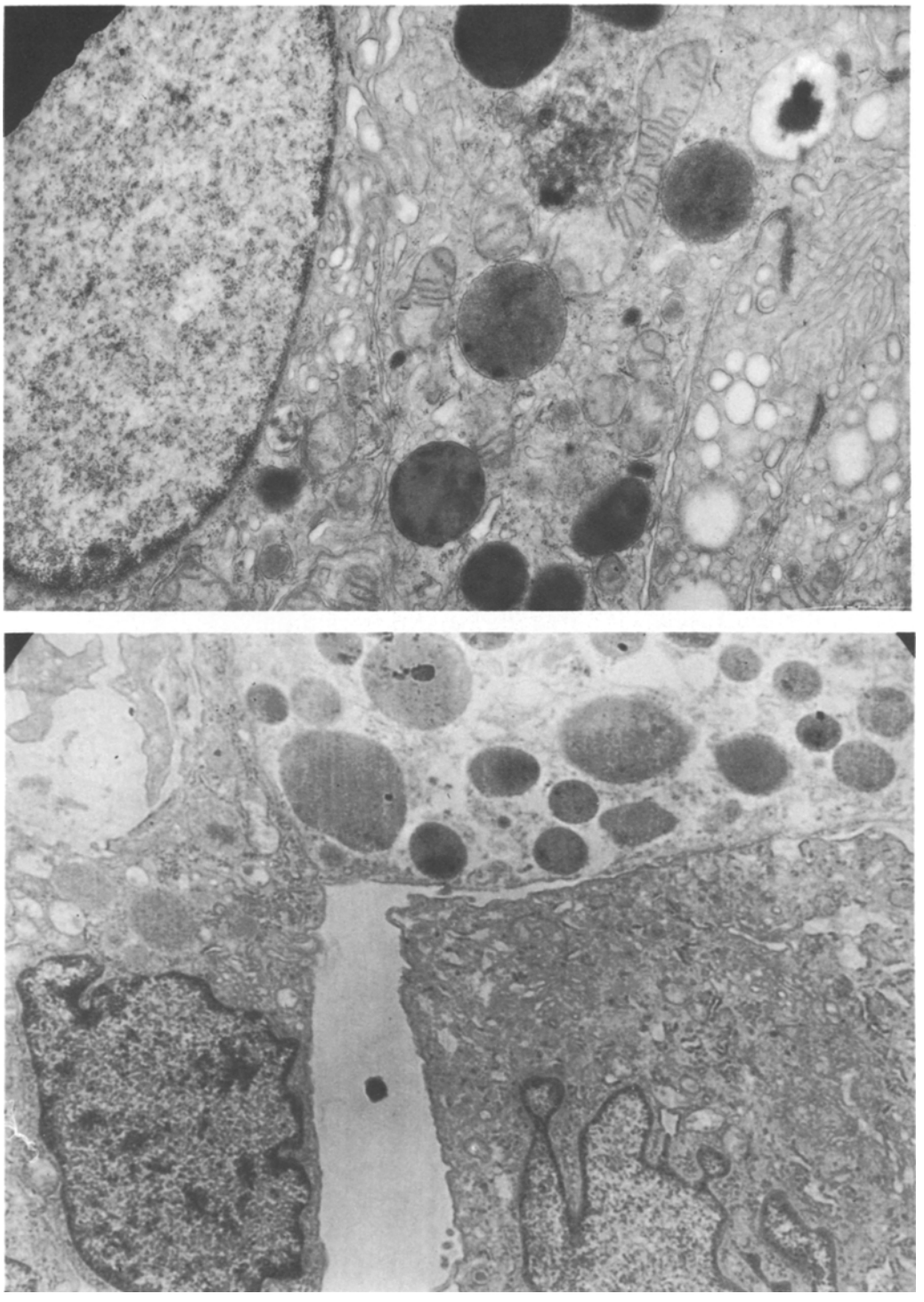

Fig. 1. a Lysosomes in BPH appear numerous in some cells and might be seen as an expression of enzymatic activity. These cells are conspicuous but quite rare. (E. M.: magnification x 10 000: Palade stain: BPH tissue). b In prostatic cancer tissue those cells do not differ in appearance, form and number from BPH tissue. With the Palade staining method no structural difference can be made at that stage due to lysosomes. (E. M.: magnification x 5000: Palade staining: prostatic cancer)

Processing tissue blocks with the Gomori staining method glutaraldehyde-fixed tissue was sliced in sections 40 µ thick on an IEC microtome cryostat, with special accessories. The sections were kept in 5% sucrose solution.

Its density was the same as that of the tissue segments prepared for the Gomori stain. Thus, the segments did not settle in the plastic tubes while the two-step embedding was carried out. The segments were packed in small blocks on a

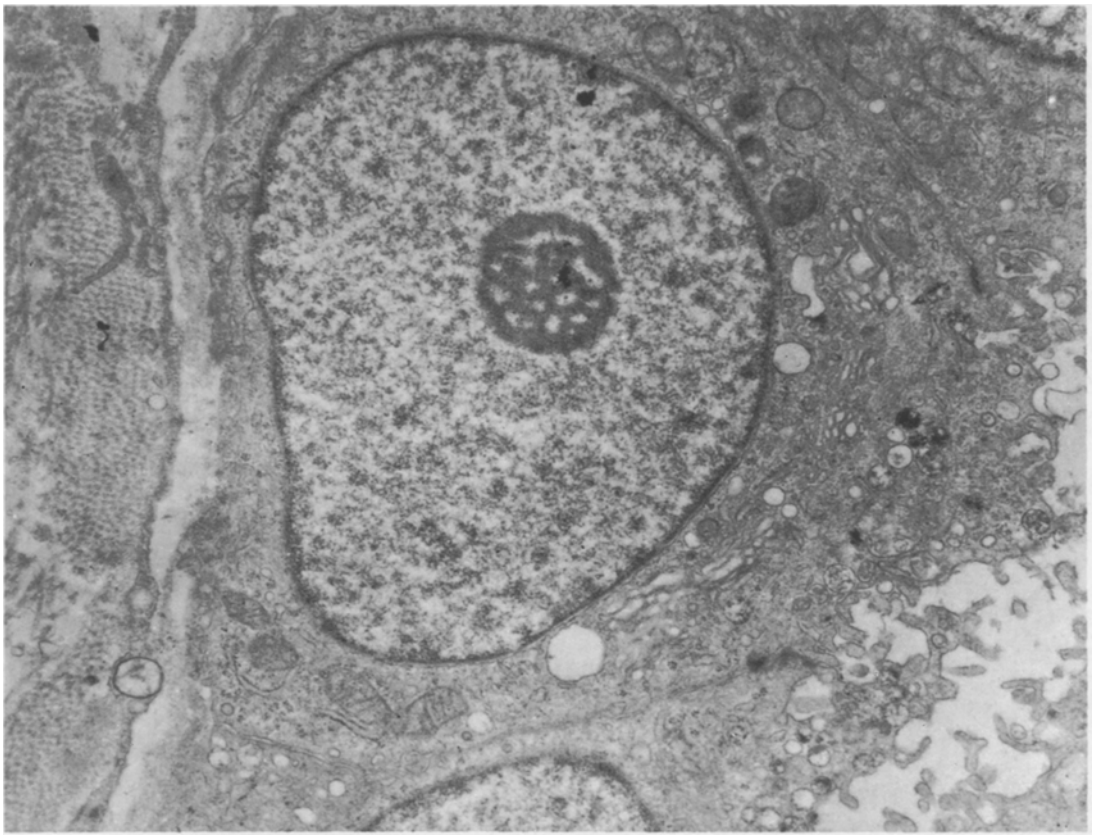

Fig. 2. Even in advanced cancer, normal-looking cells are found close to typical cancer cells. No feature here reveals the malignant character of the cell. (E. M.: magnification x 10 000: Palade staining: prostatic cancer)

plastic lid and polymerised. After polymerisation, small blocks could be embedded like the 0.5-1 mm cubes for the standard method.

All steps of the methods were carefully controlled in both types of tissues, to prevent artefacts caused by the procedures.

Gomori stain was freshly made in the standard form as a 12% lead nitrate solution (8). Dehydration and packing of tissue segments was performed as described above.

After polymerisation overnight in the case of both methods, blocks were cut and glued with Epoxy glue on prefabricated blocks, or employed for embedding in plastic caps. All tissue blocks were sliced and trimmed with glass knives on an ultra-microtome (LKB-Bromma) to a thickness of 700-800 Å.

Staining was completed with 4% uranyl acetate; in the case of the Palade stain, additionally with Reynold's lead citrate; in that of Gomori, without it. The staining procedure was performed under a petri dish with NaOH tablets to avoid carbonate precipitation. Final rinsing was done with 0.2 M NaOH.

All the photographs are taken either with a Siemens Elmiskop Model I A or a JEOL 100 C Electron Microscope.

RESULTS

The same investigations were performed in the 4 cases of prostatic cancer and the 8 benign prostatic hyperplasias. The conditions described for embedding were essential for reproducibility of results. Using the Palade staining procedure, lysosomes did not appear systematically different in form and number in benign prostatic hyperplasia and in cancer cells. We found in 2/4 samples of the cancer tissue and in 2/8 blocks of the BPH tissue particular cells with an increased number of lysosomes. Although these cells were rare in the tissue, we found them in either cancer and/or BPH tissue (Fig. 1a + 1b).

There was no evidence in this small series for the idea that hormone dependence in cancer tissues could express itself by variations in the number of lysosomes. Also no relationship was found between the number of lysosomes and the activity of acid phosphatase in the serum, as has been claimed elsewhere (18).

In the early stages of prostatic cancer, as well as in advanced undifferentiated carcinomas, cells appearing normal have been reported to occur adjacent to undifferentiated cells (13); this we have also found (Fig. 2). In the cases of BPH and ad-

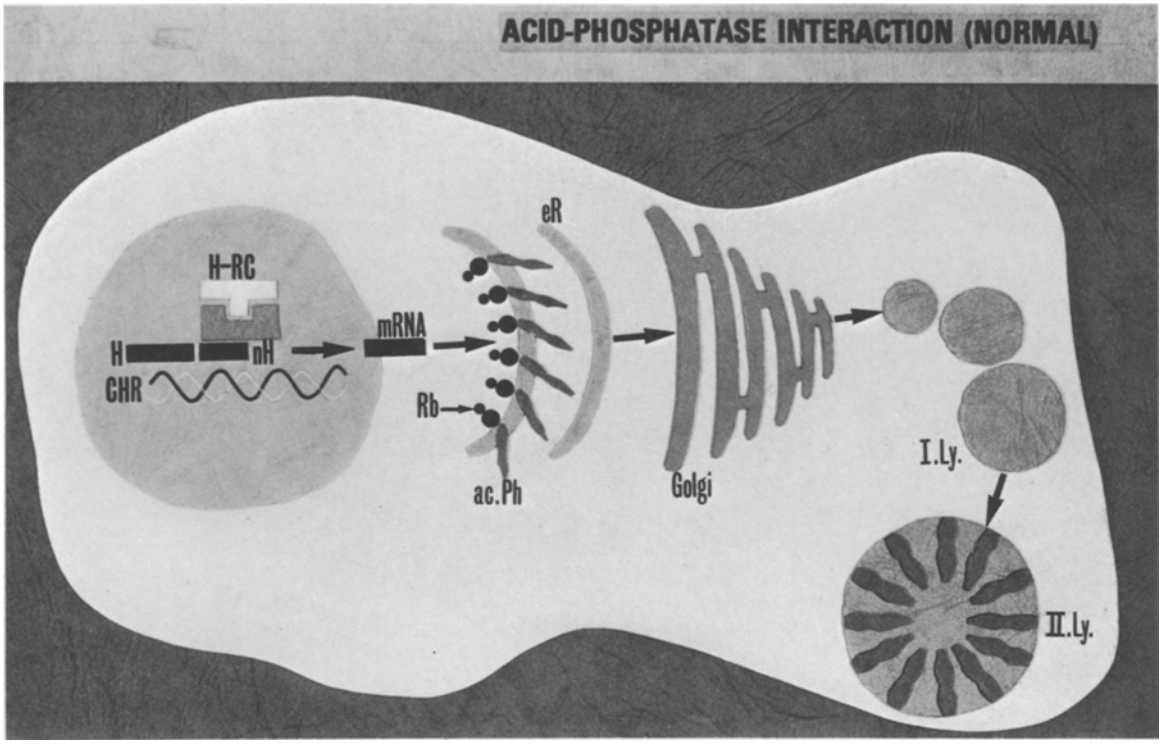

Fig. 3. Acid phosphatase (ac. Ph.) is a lysosomal enzyme which is strongly localised to the primary (I) and secondary (II) lysosomes, as well as the Golgi apparatus. The synthesis of the enzyme is probably controlled by binding of a hormone receptor complex (H-RC) to chromatin (CHR) consisting of DNA, histone (H) and non-histone (nH) components. A messenger RNA (mRNA) carries the specific genetic information for enzyme synthesis on ribosomes bound to the endoplasmic reticulum (eR). The synthesized enzyme is transported through the Golgi apparatus and packaged into the primary lysosomes (I. Ly) where it is retained under physiological conditions. Lysosomes originate from the Golgi apparatus (hetero-lysosomes) and possibly also from the cytoplasmic membranes (auto-lysosomes)

vanced cancer we investigated, it was not possible in each of the investigated blocks to make a decision as to the benign or malignant nature of the cells examined, as long as the Palade stain was employed.

To understand the pathway and distribution of acid phosphatase isozymes in BPH (Fig. 3) and prostatic cancer (Fig. 4), we used a histochemical procedure to stain the enzyme wherever it appeared in the cells. Acid phosphatase isozymes are lysosomal enzymes (17). We used β-glycerophosphate as substrate and found the reaction product restricted to the inner aspect of lysosomal membranes, while little reaction products appeared in the Golgi apparatus and the endoplasmatic reticulum. In our investigations on cancer tissue using the histochemical staining procedure, staining reactions on the nuclear membrane (Fig. 5), in - and outside the plasma membrane (Fig. 6), divergently in the cytoplasm and most of the cell organelles, as well as proteolytic damage of cell organelles (Fig. 7) was observed. None of these aberrations, nor the proteolytic damage, could be brought into relation to the level of acid phosphatase activity in the serum. None of the described histopathologic features could be seen in any of the BPH grids.

The Gomori staining method involves an incubation at 37°C, and in cancer tissue released enzymes appear to cause degradation of cell organelles. In BPH tissue such degradation of organelles was not seen, since no extra-lysosomal enzymes could be seen. Two functional aspects were thus revealed by the histochemical method: changes in the localization of the lysosomal enzymes normally restricted to these particles, and the destructive proteolytic effect of subcellular structures. In the cases of cancer, an intense reaction in the membranes and in the cytoplasm was often observed, especially if the sample was not exposed to lead nitrate in the final staining procedure.

In one case, intense nuclear staining was seen (Fig. 5). These changes were common enough to indicate that they represented typical changes occurring in cancer cells. They were never seen in BPH cells or in normal tissue.

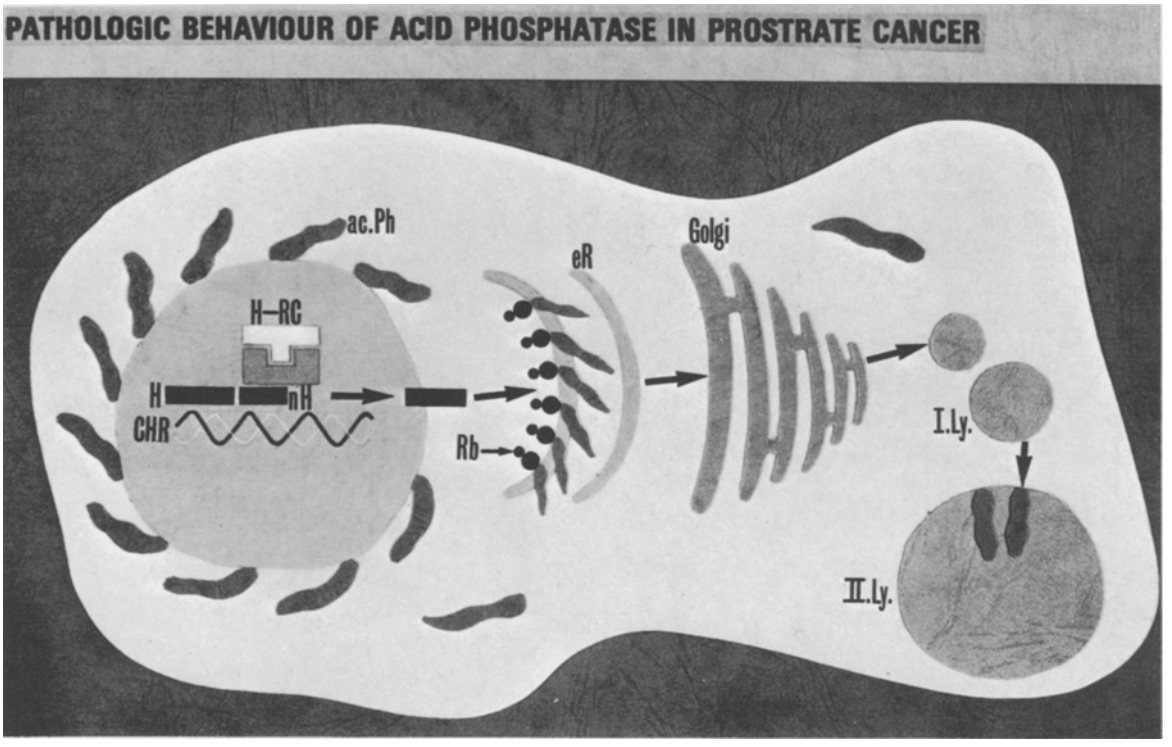

Fig. 4. Under pathologic conditions, the acid phosphatase staining reaction can be seen outside the lysosomes: on the nuclear membranes, the cell organelles (explanation as in Fig. 3) and diffuse in the cytoplasm

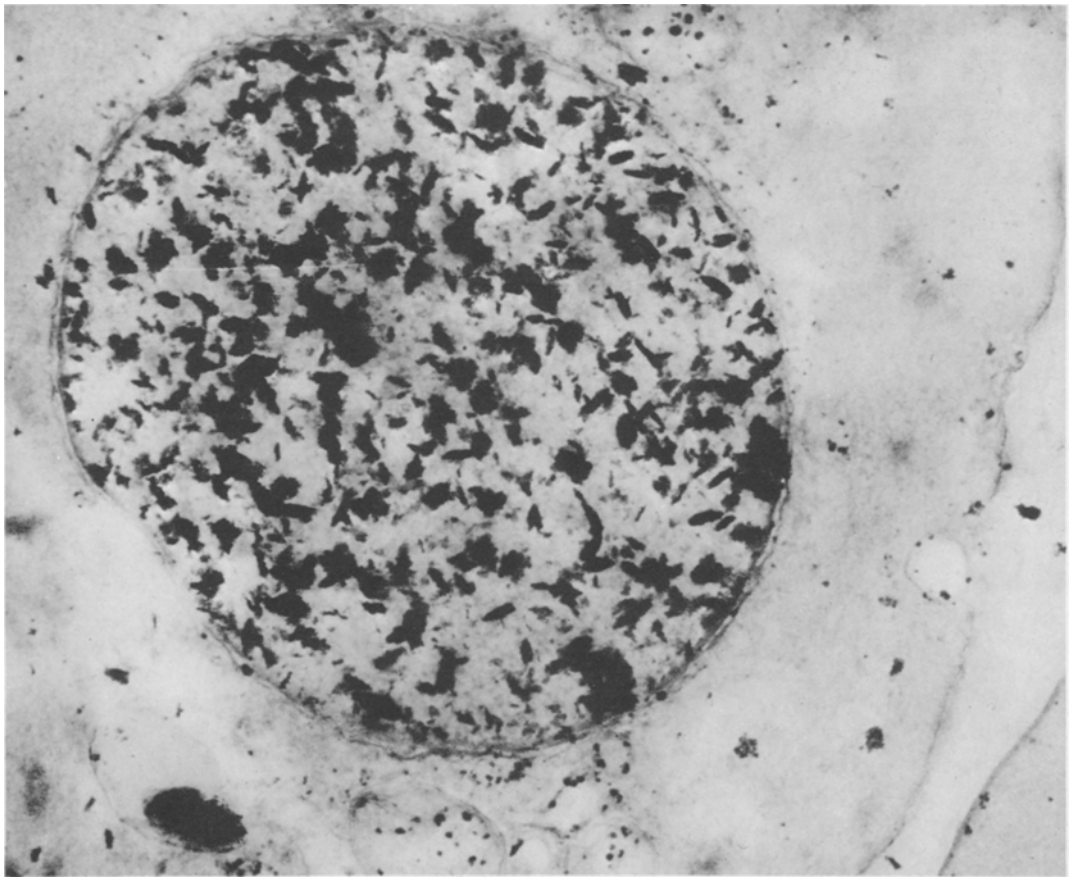

Fig. 5. This cell was present in the same tissue biopsy as shown in Fig. 2. The specific acid phosphatase staining method of Gomori revealed extensive precipitation of stain in and on the nucleus. (E. M.: magnification x 12 000: Gomori stain: prostatic cancer)

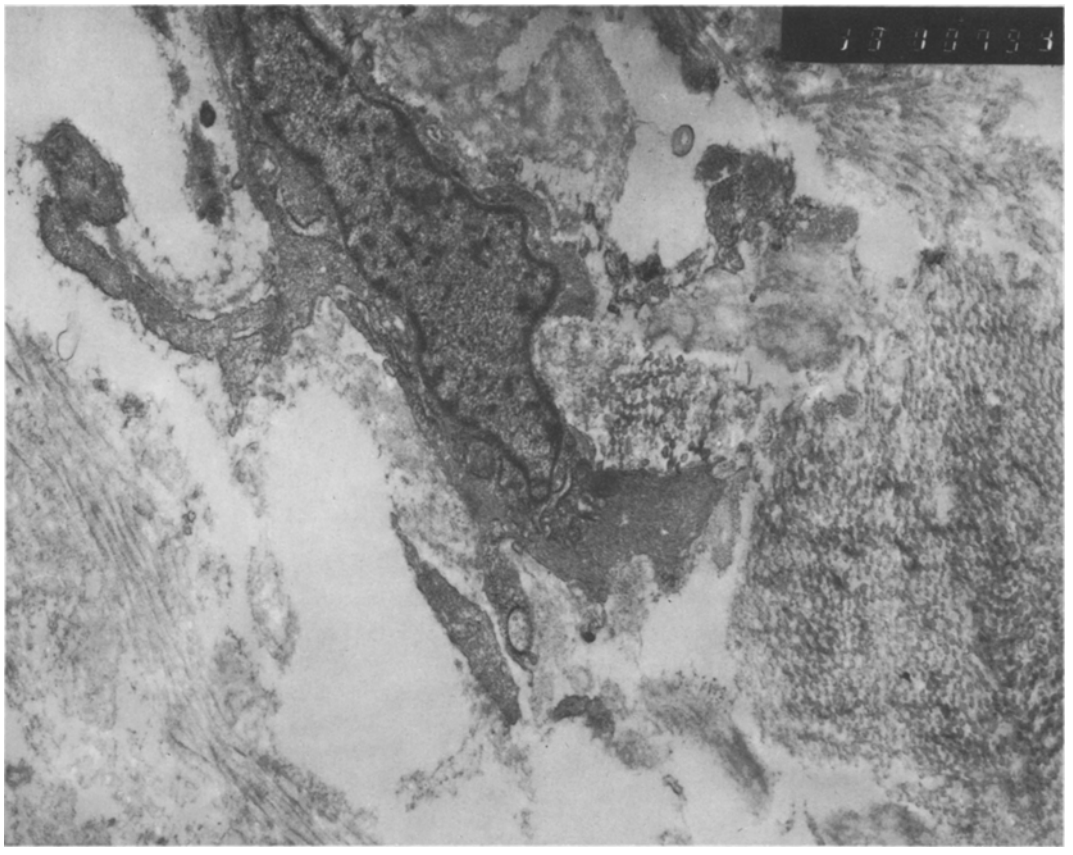

Fig. 6. In this cell from prostatic cancer tissue, a more intense acid phosphatase staining reaction was observed at the cytoplasmic membrane than in a sample of BPH tissue subjected to the same conditions. The appearance of stain is specific for extra-lysosomal acid phosphatase if no lead nitrate is involved finally in the procedure. (E. M.: prostatic cancer material: Gomori stain: magnification x 8800)

DISCUSSION

Clinical experience has shown that serum tartrate-inhibitable acid phosphatase activity is increased in certain cases of prostatic cancer. Ultrastructural investigations, however, do not indicate that this laboratory finding in each prostatic tumour is associated reproducibly with a particular pattern of change. Morphologically normal-looking cells often appear to be attached to cancer cells, in biopsies. Our histochemical investigation with respect to acid phosphatase showed that while the staining product was exclusively associated with lysosomes in all the benign cells, in cases of cancer the phosphate product was divergently and irregularly associated with various cell organelles. Thus a biochemical change in the lysosomal membrane may have occurred, which allowed the enzyme to leak out. Neoplastic plasma membranes appear to be more leaky than those of normal cells (23). The presence of acid phosphatase in the serum represents lysosomal enzyme which has leaked through pathological membranes.

A redistribution of acid phosphatase in cell cultures, other than prostatic cells, has been described (2, 5, 14). With our histochemical investigations, we have shown this phenomenon in the passage of enzyme from the lysosomes into the extracellular space.

By the histochemical procedure of staining for acid phosphatase, functional changes in the lysosomal membranes can apparently be detected at stages when structural changes in the cell are not visible. Therefore the value of these ultrastructural investigations has to be seen more in the appearance of free acid phosphatase than in the morphological structure itself. The value of the results is determined by conditions like substrate used for the staining procedure, the osmotic resistence of the tissue, dehydration, behaviour to the embedding material and the temperature used for the reaction.

With β-glycerophosphate as substrate, the reaction product of the staining procedure was restricted to the inner aspect of lysosomal membranes, while little reaction product appeared in the Golgi complexes and the endoplasmic reticulum, in agreement with previous reports (15).

Using substrates like naphthyl-phosphate (14) or naphthyl AS-B phosphate (4), or hexazonium para-

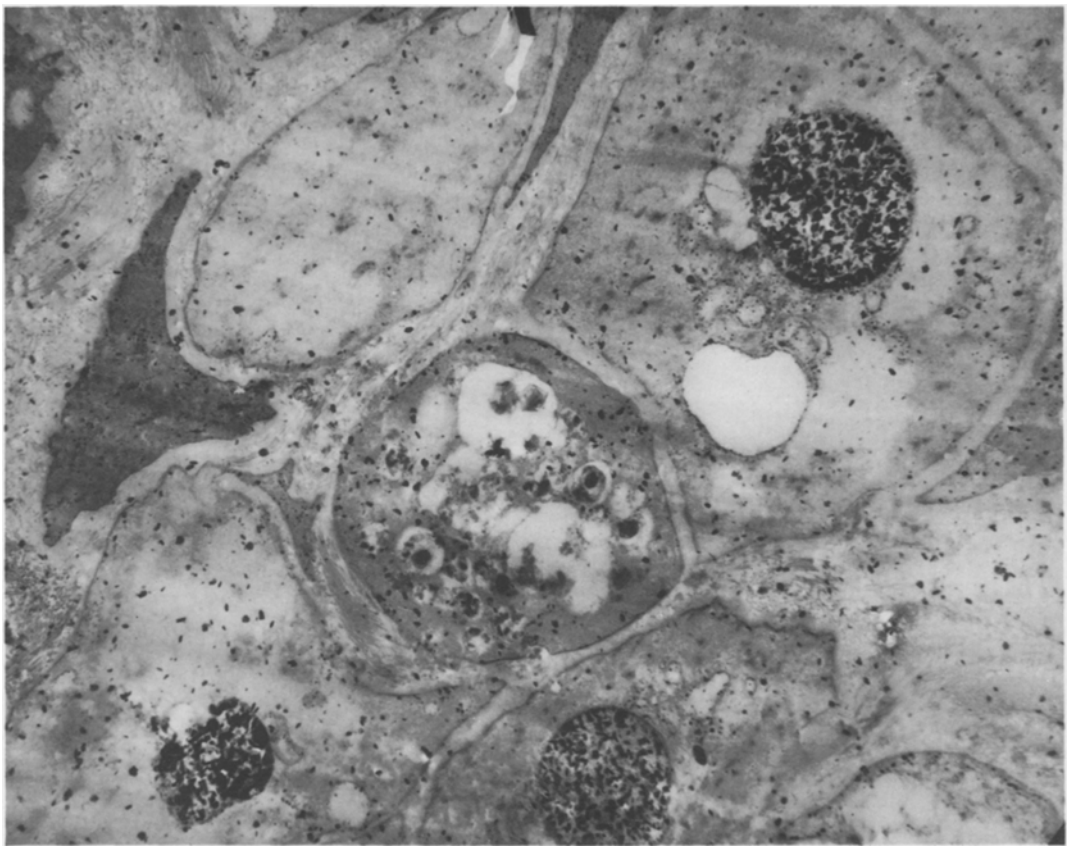

Fig. 7. The tissue is again the same as in Fig. 5. The nuclei of the whole area show acid phosphatase staining reaction as well as chromatin fragmentation. All the cell organelles are disrupted, which might be due to the proteolytic activity of released lysosomal enzymes. (E. M.: magnification x 3880: Gomori stain: cancer of the prostate)

rosaniline phosphate (1), small amounts of free acid phosphatase have been detected in the cytoplasm.

Another reason for the choice of β-glycerophosphate as a substrate for the histochemical investigation of acid phosphatase is the fact that it is more vigorously attacked than the other substrates under normal conditions (8). According to the literature (12) we preferred glutaraldehyde buffer (as described in Material and Methods) to formalin because the latter makes lysosomal membranes labile and permits the release of lysosomal enzymes (3, 7, 10). During the staining procedure, the tissue has to resist the osmotically-active agent in which it is placed. It has been shown that higher osmolarities inactivate acid phosphatase while it is activated at low ionic strengths (23).

Ethanol was used without interference in the staining procedure, as is observed using chloroform-dehydration (22).

For block embedding we found Spurr's Resin (ERL 4206) (12) to be preferable to Epon (21). The low-density polyacrylamide penetrated more easily into the tissue, while in the latter material incomplete fixation resulted. Processing the Gomori staining procedure during the necessary incubation at 37°C, activation of liberated enzyme might have taken place, which is probably due to the degradation of subcellular structures.

Even with careful observation of the various physical irritations, the electron microscopical investigation is limited to qualitative value. A quantitative result is problematic for the small area selected within each block and the variety of cell structures layered on the grid.

We see the histochemical investigation of prostate tissue as a step in the early detection of cancer even when light microscopic and other types of electron microscopic investigations are normal.

No definite relation was found between the nature of the histopathological changes and the level of acid phosphatase activity. The number of cells showing pathological changes can perhaps be seen in relation to the serum activity; although leakage may cause a complete drainage of acid phosphatase resulting in poor reaction intracellularly and high activity in the serum. From the fact that serum activity in BPH cases is low while it is high in prostatic cancer patients (opposite to the reaction in prostate secretions) cancerous change can perhaps be seen as a transformation of the lysosomal and plasma membranes. This leads to

the paradoxical distribution of the acid phosphatase activities.

Artefactual staining was excluded by parallel staining in benign and malignant tissue with various concentrations. Even when the final washing was incomplete, none of the described aberrations could be seen in BPH tissue.

While ultrastructural examination with the Palade stain did not solve the general problems encountered in histological investigations the additional functional and biochemical characterization permitted by enzyme histochemistry appeared to be useful.

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