BRIEF COMMUNICATION

MULTIPLE FORMS OF PROSTATIC ACID PHOSPHATASE

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Summary. Human prostatic fluid and prostate extract were examined for prostatic acid phosphatase by means of gel diffusion precipitation, using rabbit antisera. Specific staining for phosphatase was performed on the antigen–antibody precipitation lines. Three major and two minor bands, staining for phosphatase, were detected: one of the major bands was shown to correspond to a non-specific precipitate. Of the four specific precipitates, one was found only for fluid, but not for extract. The staining of all of the precipitates was inhibited by the presence of tartrate, confirming the identity of the antigen as prostatic-type phosphatase.

It has been known for some time that prostatic fluid is very rich in acid phosphatase activity (Mann, 1964). While similar enzymatic activity is also found in other body fluids and in tissue extracts, the prostatic variety is considered distinctive because it is completely inhibited by low concentrations of tartrate ion (Jacobsson, 1960). Several types of prostatic acid phosphatase have, however, been reported, and the precise degree of correlation between them is not clear. This may be due in part to undetected polydispersity of one or more types of preparation. Our studies on prostatic acid phosphatase, using immunochemical means of analysis, have, in fact, revealed a considerable degree of molecular heterogeneity.

Earlier investigations had shown that this enzymatic activity could be directly detected and analysed in human prostatic fluid by the gel diffusion method of antigen–antibody precipitation (Mamrod, Shulman, Gonder & Soanes, 1964; Shulman, Mamrod, Gonder & Soanes, 1964, 1965). By special staining procedures, taking advantage of the enzymatic nature of the antigen, the phosphatase was localized in a dense, broad, brown band and, in some cases, in a second, less conspicuous, broad band, which was much closer to the antigen well. The total procedure consists, in essence, of two steps, the first being the antigen–antibody precipitations, and the second being the enzymatic staining procedure. The details of technique have been fully described (Shulman et al., 1964). It need only be emphasized here that it is essential to select the antiserum to be used with great care.
In the current investigation, human prostate extract was studied, as well as prostatic fluid, and a new group of rabbit antisera, consisting of a series of bleedings from each of several animals, was explored. Each successive bleeding is indicated alphabetically. The extracts were prepared from random tissue, obtained at autopsy, which was homogenized with an equal mass of saline (0.15 M NaCl), and then clarified by centrifugation. Several samples were pooled. The fluid was collected from a number of individuals, by methods previously described (Soanes & Brodie, 1960), and pooled. Protein concentration (in percentage) was estimated by the biuret method (Gornall, Bardawill & David, 1949), using an extinction coefficient of 2.8 at 540 m\(\mu\), a value determined in this laboratory for a sample of pooled human serum. Initial experiments showed that prostate extract is just as suitable for showing the enzymatic staining, as is the prostatic fluid. Several major and minor lines could be readily seen for phosphatase. By comparing serum samples taken at various times from each of several rabbits under immunization with human prostatic fluid, it was soon realized that five distinctive lines of precipitation, staining for phosphatase activity, could be observed. Plate 1 illustrates two sets of typical patterns, using a selected bleeding from rabbit 226 and from rabbit 227. Both the extract and the fluid were tested at 1.0% and 0.1% protein. Three heavy bands were seen, as well as two light and fine lines. All of these were brown in the original plates.

From study of many experiments of this type, some general characteristics can be described for the individual stained precipitates. There are three major bands. One of these is a short, broad band on the edge of the antigen well and concave to it. This was often, but not always, accompanied by a stained ring, or 'halo' around the antigen well; this depended on the selection of antiserum. The halo was finally shown to be a non-specific precipitation, even though it did not occur around the wells containing serum or saline. An alternative plate was set up containing these extracts and fluids, but having normal rabbit serum in place of an antiserum. In this plate, the halos were seen around the wells, just as with antiserum, but none of the other lines was present. In Plate 1 both of these bands can be clearly seen for the concentrated prostate extract as antigen. The additional major stained precipitate is generally seen as a long, broad dense band, located midway between the antigen and antiserum wells, or even closer to the antiserum well, and concave to it. In Plate 1, it is seen as the most conspicuous feature of the patterns. In addition to these strong bands, some faint lines can be detected. They are interesting because they remain very thin and sharp. One of these is often seen on (or just beyond) the outer edge of the major line, mentioned above. It is readily seen in Plate 1, most clearly for antiserum R 226 i, and equally well for prostate extract and fluid. Lastly, a fine line is found to be characteristic for the fluid, and is not seen for the extract. This is best shown for R 226 i in Plate 1, although it is visible in the other experiment also. Other investigations in which prostatic fluid and extract were placed in adjacent wells have shown that this line does not correspond to any component detectable in the extract.

These five stained lines of precipitation (representing four specific antigenic components) have been seen repeatedly in such studies, although occasionally
Double diffusion gel precipitation, stained for acid phosphatase. Reactions carried out in 1% agar in saline (0.15 M NaCl), using a micro technique.

Wells are 2.5 mm in diameter. Distance between wells is 4 mm, edge to edge. Plates were incubated for 24 hr at room temperature, washed in saline for 2 days, and dried on glass plates. Development of the stain was accomplished by incubating the dried gels for 10 min at 37°C in a 10:1 (v/v) mixture of buffer, 0.05 M acetate, pH 5.0, containing lead nitrate, 0.13%, and substrate, β-glycerophosphate, 3%. Following incubation, the gels were washed in 1% acetic acid, and then immersed in 2% yellow ammonium sulphide. The stain appears as a dark brown precipitate of lead sulphide.

The contents of the wells were as follows. Antigens: Well (1) human prostatic extract at 1%; (2) prostate extract at 0.1%; (3) saline; (4) human prostatic fluid at 0.1%; (5) prostatic fluid at 1%; and (6) normal human serum at 2.5%. Antisera: Centre well (a) R226 i; and (b) R227 i, both rabbit anti-human prostatic fluid.

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one may be missing, depending on the samples used. All of them fail to become stained if L-tartrate (0.01 M) is included in the buffer-substrate solution during the staining procedure (Mann, 1964). Thus, all seem suitable for labelling as 'prostatic acid phosphatase.' Studies are currently being made on the separation of these phosphatases by chromatography on DEAE-cellulose, using the appropriate antisera as test reagents. It may be tempting to label these enzymatic forms as isoenzymes, but it would seem premature to do so at this time. It should be considered essential to show that they are, in fact, molecularly related, before this terminology is applied. Studies along these lines are in progress. It does seem clear, however, that four or five different forms of acid phosphatase enzyme exist in prostatic tissue and secretion.

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