Prostate Antigens and Antibodies[1] (35520)

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Earlier studies of prostatic tissue extract or prostatic fluid have shown the presence of several prostate-specific antigens and autoantigens. This property of tissue specificity has been demonstrated for the human (1–5), canine (6–9), and lapine (10–12) systems. In addition, the rabbit prostatic tissue has been shown to contain autoantigens (11, 12). The molecular nature of this antigen has not been clarified, although a few details are known about the major proteins of these fluids or extracts. It would thus be of interest to purify the individual antigens and then to study their antigenic and autoantigenic properties. For this goal, the fluid secretion would seem to be a better starting material than a tissue extract; among these possibilities, the canine secretion was of special interest, because it is more homogeneous than the human fluid and it can be obtained in large quantities (13).

The present report describes some preliminary success in the fractionation of canine prostatic fluid (CPF) and some observations on the several separated antigens.

Materials and Methods. Prostatic fluid. This secretion (CPF) was obtained from dogs that had been surgically prepared by the method of Mason *et al.* (14), on injection of pilocarpine. Most of the material was kindly given to us in freeze-dried form by Dr. Harris Rosenkrantz of Mason Research Institute, Worcester, Mass. Dog serum was obtained from several intravenous bleedings.

Antiserum. Several New Zealand White rabbits were injected with CPF, incorporated with Freund's complete adjuvant.

Protein fractionation and measure of concentration. A sample of CPF, about 200 mg, was placed on a column of Sephadex (2.5 × 100 cm) that had been suitably swollen, poured, and packed. The equilibrating and eluting fluid was plain saline (0.15 *M* NaCl). The eluted fractions were measured for concentration by obtaining readings of optical density at 280 mμ in a Beckman DU spectrophotometer. Appropriate pools of fractions were assembled, in some cases made more concentrated by means of ultrafiltration, and concentration was measured by use of the biuret method, with readings at 540 mμ using the spectrophotometer.

Gel diffusion. Suitable wells were prepared in 1% agar (in saline) in disposable petri dishes (50-cm diam), at several well sizes and separations. Diffusion was followed for several hours and days, and patterns of interest were sketched or photographed.

Ultracentrifugal methods. Experiments were performed in the Spinco Model E machine.

Results. The initial CPF showed several lines of precipitation in gel diffusion with rabbit antiserum. By selecting the best antiserum sample, a pattern of three lines could be seen. A typical picture is shown in Fig. 1.

Fractionation was attempted on a column of Sephadex G-100. A typical elution graph is shown in Fig. 2. A group of three major peaks was seen. The fractions were assembled into pools, and these three pools were designated 1, 2, and 3, in order of elution. The analysis of yields indicated that pool 1 was about 1%; pool 2, 9%; and pool 3, 90%, of the total recovered protein. Each of these pools was examined in the ultracentrifuge and a homogeneous appearance was given by 2 and 3 at the concentrations available for examination; pool 1 was too limited in amount for study. The sedimentation coefficients for pools 2 and 3 were 4.0 S and 2.6 S, respectively, compared to a value of 2.5 S for the whole CPF. Pool 3 then seems to be the major component seen in CPF, whereas pool 2 seems to be a different substance, and one that is not detectable in the ultracentrifugal

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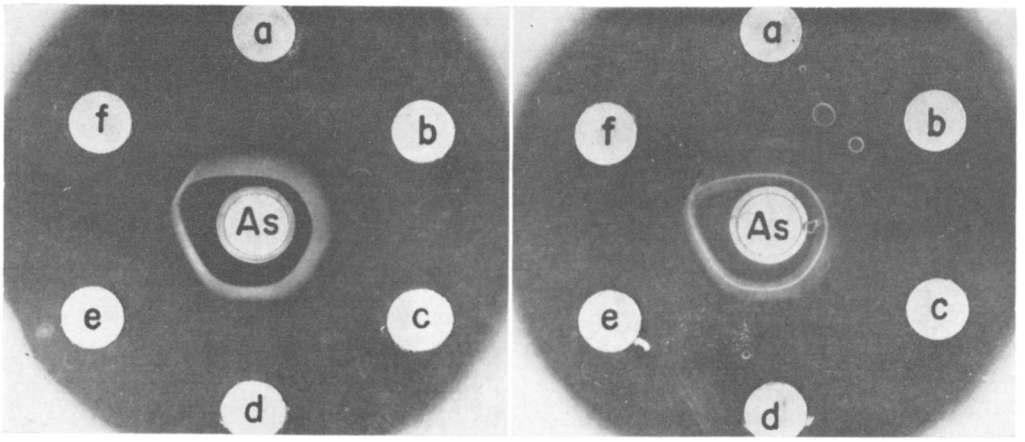

FIG. 1. Double diffusion gel precipitation: central well, anticanine prostatic fluid (R62 final); peripheral wells, CPF (mg/ml): (a) 80.0; (b) 40.0; (c) 20.0; (d) 10.0; (e) 5.0; (f) 1.0. (left) early (16 hr); (right) late (24 hr).

analysis of whole CPF.

Antigenic studies were made of pools 2 and 3, using the same antiserum as before. The reaction for pool 2 consisted of two lines of precipitation; one of them was quite dense and the other was quite faint, but both were sharp and definite. We shall mainly consider the more intense line in the present report. The reaction for pool 3 consisted of two (eventually) rather faint and somewhat broad lines of precipitation, but they cannot be seen in each experiment, since factors of total concentration and time of diffusion influence the appearance. An early-appearing line fades after a few hours, and it has not been much studied. The second line is seen only with higher concentrations of pool 3. A picture of such reactions is shown in Fig. 3, where the early-appearing line of pool 3 is not seen. An additional feature can also be noted in Fig. 3. There is an apparent shortening of the more intense line seen for pool 2,

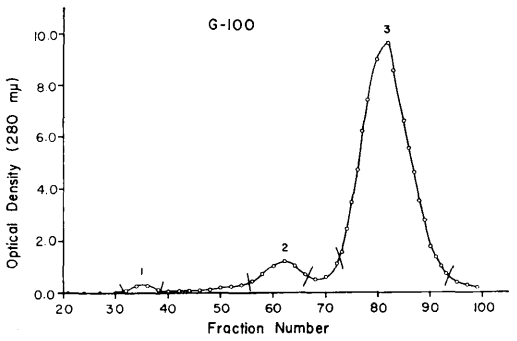

FIG. 2. Elution pattern of canine prostatic fluid on Sephadex G-100: The sample was 8.2 ml of canine prostatic fluid, having a protein concentration of 30.0 mg/ml, and was applied to a gel bed of 2.5 × 100 cm. Eluting solvent was 0.15 *M* NaCl. Fractions were collected every 12 min and they averaged 3.0 ml in amount. The pools were grouped as indicated.

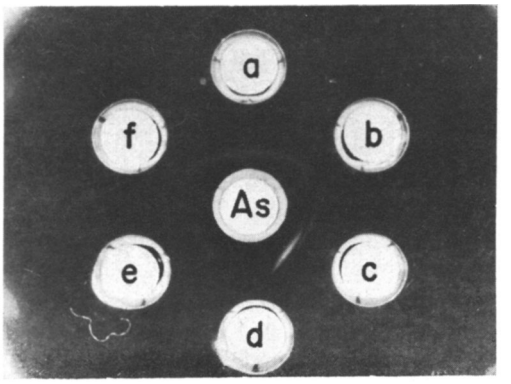

FIG. 3. Double diffusion gel precipitation: central well, anticanine prostatic fluid (R62 final); peripheral wells: (a) CPF, 80.0 mg/ml; (b) pool 3, 51.0 mg/ml; (c) pool 2, 2.0 mg/ml; (d) saline; (e) pool 3, 51.0 mg/ml; and (f) saline.

on the side near the well containing pool 3. This observation suggested that a kind of inhibition was provided by a component of pool 3, which interfered with the precipitation of a component of pool 2. To verify this impression, a comparison plate was set up, as shown in Fig. 4. This experiment shows

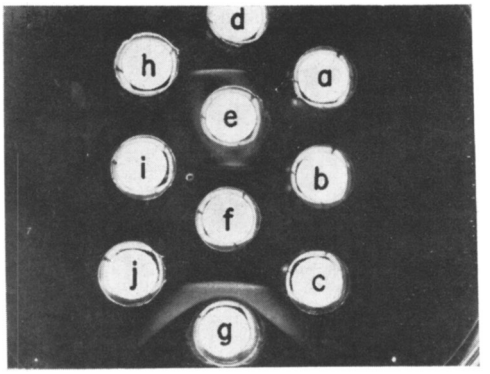

FIG. 4. Double diffusion gel precipitation: Pools 2 and 3 were tested at protein concentrations of 1.0 and 7.6 mg/ml, respectively: (a) pool 3; (b) pool 3; (c) saline; (d) pool 2; (e) anticanine prostatic fluid (R62 final); (f) pool 2; (g) anticanine prostatic fluid (R62 final); (h) saline; (i) pool 3; (j) saline.

three versions of the precipitation between pool 2 and antiserum; in one version, pool 3 is in a well at one end of the line, in another version, it is at both ends, and in the third, it is absent from both ends.

Discussion. Earlier studies showed that canine prostatic fluid was rather paucidisperse, with more than 90% of its protein content in one main ultracentrifugal component with a sedimentation coefficient of 2.7 S and a very small peak with a rate of 16 S (6, 13); in an occasional sample, a small component with a rate of 4.2 S could be seen (13). Electrophoretic analysis revealed one main component, of mobility like that of serum γ-globulin (13). Antigenic analysis had shown that prostate-specific material was present, as clearly indicated by passive hemagglutination with absorbed antiserum and by gel diffusion. There was a reaction with extract of prostate gland and with seminal plasma, but no reaction with other tissues, such as testis, bladder, kidney, liver, spleen, heart, muscle, or brain. Some of the other antigenic activity, in contrast, was shown to be due to serum antigens, but these would be readily distinguished from the principal, specific antigens (6).

It was now found that three major pools or components could be isolated from canine prostatic fluid. These pools were moderately homogeneous, but each one must have contained several constituents. The major antigen in pool 2 seemed to be the same as the most strongly precipitating antigen of whole CPF, despite the fact that it is a small proportion of the recovered mass of protein. It also had a sedimentation coefficient of 4.0 S, which corresponded to a generally undetectable component in the ultracentrifugal pattern. This need not be taken to be serum albumin, since preliminary electrophoretic analyses of this pool showed that albumin was at best a minor component. In addition to this, we have definite evidence from gel diffusion experiments, that the faint lines of pools 2 and 3 represent antigens common to normal canine serum and that the main lines of these pools are definitely not shared by the antigens of canine serum.

The major antigen of pool 3 is a predominant part of whole CPF, both by the criteria of gel filtration yields and by its sedimentation coefficient of 2.6 S.

The reasons that pool 3 inhibits the precipitation of pool 2 are not yet clear. It is also not clear as to why this inhibitory interaction does not seem to occur in the reaction of whole CPF. One possibility is that pool 3 contains a low molecular weight substance with antigenic sites similar to those on the comparatively high molecular weight antigen of pool 2. These low molecular weight substances would provide the inhibition by interfering with the precipitation of one of the antigens of pool 2 and its antibody. If such substances occur, one must ask where they originate, whether in biosynthesis of the CPF components or from a proteolytic digestion caused by enzymes that are present in this fluid (15, 16).

Summary. It was shown that canine prostatic fluid (CPF) could be fractionated into three components by gel filtration on Sephadex G-100. These components were referred to as pools 1, 2, and 3 in order of elution and their relative proportions were 1, 9, and 90%, respectively, of the total recovered protein. The sedimentation coefficients for pools 2 and 3 were 4.0 S and 2.6 S, respectively. Whole CPF, on the other hand, showed only one peak of 2.5 S.

Antigenic analysis of whole CPF, in gel

diffusion, showed three lines of precipitation when tested against rabbit anti-CPF. Reactions of pools 2 and 3 consisted of two lines each; one faint one and the other more intense and sharp. The more intense line of precipitation of pool 2 was inhibited by a component of pool 3. It was suggested that pool 3 might contain low molecular weight substance(s) with antigenic sites similar to those on the main antigen of pool 2.

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